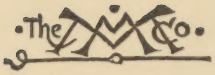
OUTLINES OF MEDICAL ZOÖLOGY



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OUTLINES OF MEDICAL ZOÖLOGY

With special reference to laboratory and field diagnosis

BY

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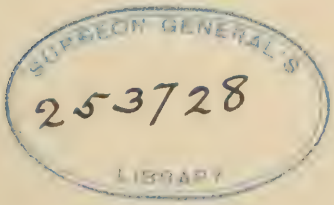

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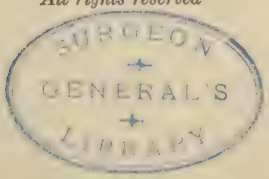

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PREFACE

Due to the favorable reception accorded the bulletin published in 1921, entitled "Diagnosis of Protozoa and Worms Parasitic in Man," the authors have felt impelled to revise and in some cases entirely rewrite some of the topics with the idea of furnishing a brief textbook or Outlines of Medical Zoölogy for the use of public health officers, students and physicians (especially those practicing in tropical or semi-tropical countries). In attempting to accomplish this aim, new material has been added; as, for example, a section on arthropods.

As in the first edition no attempt has been made to include in the keys and descriptions all of the species that are now known. On the contrary, species that have been recorded only once or a very few times have usually been purposely omitted to avoid confusion.

The information contained in this textbook is based on the personal experiences of the writers or selected from original articles in periodicals, and from reference books and textbooks wherever available. Credit for the use of figures is given in every case in the descriptions of the figures. A few of the more important books and articles relating to the parasites and arthropods described are listed at the end of the

account of each group. Other lists will be found in the bibliographies contained in most of these books and articles. So far as possible we have verified statements regarding the organisms described, but errors are almost certain to creep into such a compilation, and we will welcome corrections. We will also be glad to accept any suggestions for the improvement of the textbook which may be incorporated in a second edition if called for.

The methods employed by various workers for the diagnosis of animals of medical importance are very numerous and only a few could be described in this textbook. These have been selected on the basis of simplicity and effectiveness. It seems to us desirable that methods applicable to field conditions and requiring the minimum of apparatus and reagents should be emphasized. Modifications of these methods may be devised to fit the circumstances encountered in the field.

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BY

WILLIAM W. CORT

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GENERAL LITERATURE LIST

This general list contains titles of only a few books and periodicals selected on the basis of helpfulness and accessibility. Special lists will be found at the end of each section of the textbook.

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PART I

PROTOZOA PARASITIC IN MAN

PART I

PROTOZOA PARASITIC IN MAN *

BY

ROBERT W. HEGNER

1. INTRODUCTION TO THE PROTOZOA

THE Protozoa may be defined as unicellular animal organisms usually microscopic in size, but nevertheless exhibiting many activities similar to those of the higher animals, though in a simpler form. They are generally separated into four classes according to the presence or absence of locomotor organs and the character of these when present. The class Sporozoa contains only parasitic species, but the other three classes comprise both free living and parasitic forms. Species parasitic in man occur in all four classes. The following is a brief classification of the Protozoa, with examples of human parasites:

Class 1. Sarcodina. With pseudopodia. *Endamœba histolytica, E. coli, Endolimax nana.*

Class 2. Mastigophora. With flagella. *Giardia lamblia, Trichomonas hominis, Trypanosoma gambiense, Leishmania donovani.*

Class 3. Sporozoa. Without locomotor organs in adult stage: sporulation occurs. *Plasmodium vivax, Isospora hominis.*

Class 4. Infusoria. With cilia. *Balantidium coli.*

* The writer is indebted to his colleague Dr. W. H. Taliaferro for valuable assistance in preparing the sections devoted to hemoflagellates, amœbæ, and fecal diagnosis.

2. Blood-inhabiting Protozoa of Man

A. Organisms that Cause Malaria in Man

1. *Classification.* The organisms that cause malaria in man belong to the class Sporozoa, subclass Telosporidia, order Hemosporidia and family Plasmodidæ.

The members of the class Sporozoa are parasitic Protozoa without locomotor organs and are further characterized by the method of reproduction known as sporulation. In the subclass Telosporidia the vegetative (trophic) stage precedes and is separate from the sporulation stage. The members of the order Hemosporidia are intracellular in the trophozoite stage, have no resistant spores and undergo an alternation of schizogony in a vertebrate and sporogony in a blood-sucking invertebrate (e.g., mosquito). To the family Plasmodidæ belong the genera *Hæmocystidium* which occurs in reptiles, and *Plasmodium* which includes the malarial organisms. Three species of *Plasmodium* are known from man, (1) *P. vivax*, the organism of tertian malaria, (2) *P. malariæ*, the organism of quartan malaria, and (3) *P. falciparun*, the organism of estivo-autumnal malaria. Malarial parasites also inhabit lower animals, such as *P. danilewskyi* in birds, *P. kochi* in chimpanzees, *P. bovis* in cattle, *P. canis* in dogs, *P. equi* in horses and *P. diploglossi* in lizards. In the accompanying table (p. 17) the distinguishing features of the three species of *Plasmodium* occurring in man are contrasted as an aid in identification.

2. *Life Cycles.* The complete life cycle of the malarial parasites consists of an asexual cycle, involving schizogony, in man, and a sexual cycle, including sporogony, in the mosquito. Only female mosquitoes belonging to certain species of the genus *Anopheles* are parasitized by the malarial parasites and are able to transfer these parasites to man.

The life cycle of the tertian malarial parasite, *Plasmodium vivax* (Pl. I), will first be described with the aid of figures and then differences between this species and the other two species will be pointed out. In all three the stages are in general alike: (1) Sporozoites (Pl. I, 1), are inoculated into man by infected mosquitoes; (2) asexual multiplication by schizogony (Pl. I, 2–5), occurs in the blood of man; (3) sexual stages, gametocytes (Pl. I, 6a–6b), are also formed in the blood of man; (4) the gametocytes can develop further only in the body of the mosquito, first producing male gametes and female gametes (Pl. I, 7a–7b); (5) these undergo fertilization (Pl. I, 8), thus forming zygotes, which change to worm-like oökinetes (Pl. I, 9), and become oöcysts (Pl. I, 10–12) in the wall of the mosquito's stomach; (6) within the oöcysts many sporozoites (Pl. I, 12) are formed; some of these become located in the salivary glands of the mosquito, which is then infective to man.

a. *Plasmodium vivax.* (1) Sporozoite (Pl. I, 1). The infective stage in the mosquito is known as a sporozoite, a minute spindle-shaped cell 10 to 20

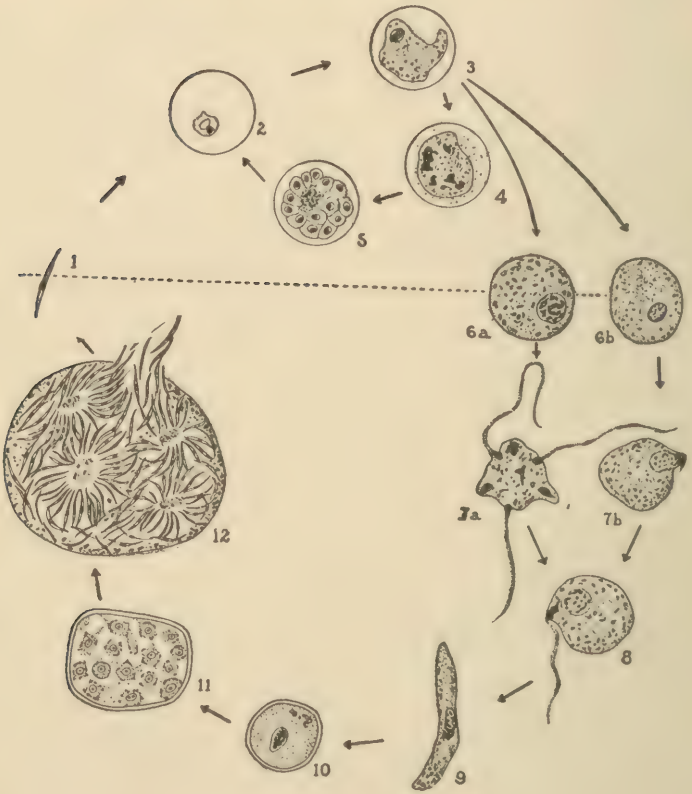


PLATE I. — LIFE-CYCLE OF THE TERTIAN MALARIAL ORGANISM, *Plasmodium vivax*.

(The stages above the dotted line occur in the peripheral blood of man, whereas those below are found only in the mosquito.)

1. Sporozoite. *2*. Trophozoite, in red cell. *3*. Full-grown schizont. *4*. Schizont with chromatin in several masses. *5*. Segmentation stage. *6a*. Male gametocyte. *6b*. Female gametocyte. *7a*. Exflagellation of male gametocyte-formation of microgametes. *7b*. Female gametocyte extruding chromatin from nucleus. *8*. Fertilization of macrogamete by microgamete. *9*. Ookinete. *10*. Young oocyst. *11*. Oocyst with sporoblasts forming. *12*. Ripe oocyst discharging sporozoites.

microns in length and 1 to 2 microns in width. These sporozoites are stored in the salivary glands of the insect. When the mosquito "bites" it pierces the skin with its proboscis and a salivary secretion containing the parasites then passes along a groove and into the wound. The number of sporozoites injected depends on the number present in the mosquito and the number of times the mosquito bites. There are perhaps not more than 10,000 sporozoites in the salivary glands of a single mosquito, and usually less than this number. That several thousand may enter the blood from a single mosquito, seems quite reasonable. Sometimes the blood cells are doubly infected, that is, more than one malarial parasite is present in a single corpuscle. This is especially characteristic of the estivo-autumnal species; it probably occurs only after several asexual cycles have caused a considerable increase in the number of parasites in the blood.

(2) Trophozoite (Pl. II, 1). The sporozoite changes by contraction into an ameboid form after it enters the blood cell, being about 2 microns in diameter. It is a hyaline body, now known as a trophozoite. In living blood the trophozoite may be seen actively throwing out and withdrawing pseudopodia within the corpuscle. The trophozoite of tertian malaria is especially active — a fact that suggested its specific name "*vivax*." The protoplasm of the blood cell is used as food by the trophozoite, and nutriment is probably also obtained from the blood stream. As growth proceeds (Pl. II, 2),

the corpuscle becomes enlarged and about 6 to 8 hours after being infected fine, reddish-brown granules begin to appear within the parasite (Pl. II, 3). These are pigment granules (also called melanin), and represent chiefly the by-products of the digestion of hemoglobin. They may be seen to move about in the parasite, due to streaming movements of the cytoplasm. In preparations stained by the Romanowsky method the young trophozoite appears ring-shaped, with a minute red mass of chromatin on one side, a central vacuole-like area, and an outer border of blue-colored cytoplasm which is thicker, usually opposite the chromatin-mass (Pl. II, 1). Older trophozoites change from the ring stage to an ameboid shape, as indicated in Pl. II, 2–5, and by the end of 36 hours almost entirely fill the blood corpuscle (Pl. II, 5). The corpuscle gradually increases in size during the growth of the trophozoite — a characteristic peculiar to the tertian infection. Granules, that stain a pinkish color, may also appear in the part of the red cell not occupied by the parasite. These are probably degenerations of the protoplasm of the parasitized cell and are termed Schüffner's granules (Pl. II, 2, 5, 10). They are characteristic of tertian infections and thus of diagnostic value.

(3) Schizogony. Trophozoites that undergo multiple division are called schizonts and the process is known as schizogony. The fully grown schizont at the end of 36 hours is from 8 to 10 microns in diameter (Pl. II, 5). Growth now ceases and the

PLATE II. — STAGES IN THE LIFE CYCLE OF THE OPALINA OF TRITONS MEMBRANA, *Diaphragm plate*, IN THE *Rana* & *Nyn*.

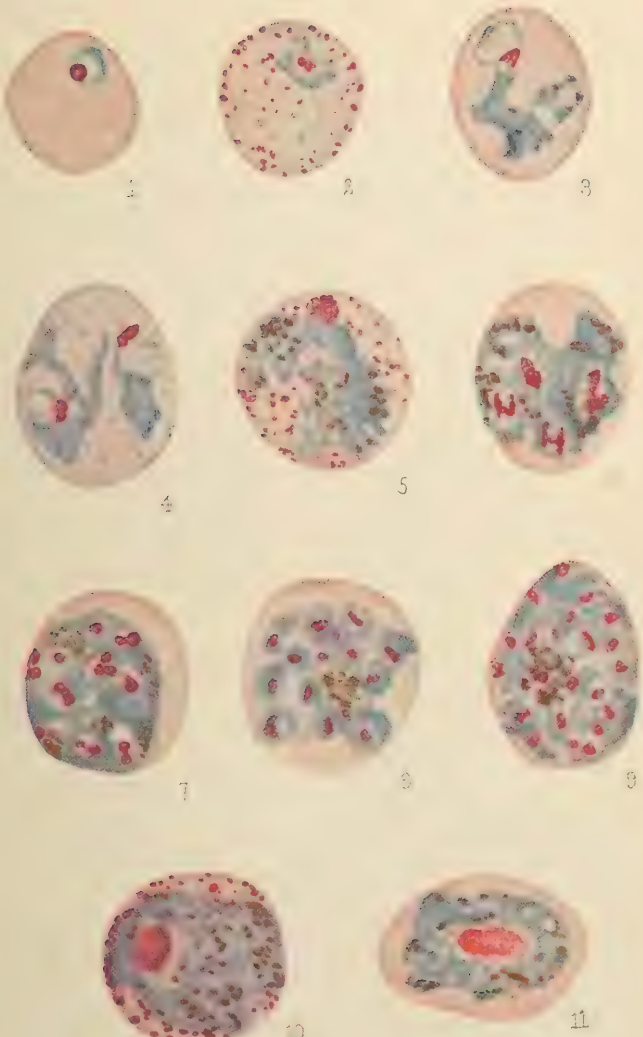
(All drawn under the author's direction by Miss E. for Sorts from original prepara-
tions at a magnification of 1500 diameters)

1. Trophozoite in resting stage. 2. Slightly older trophozoite, with amoeboid shape-
changes of body and beginning of division. 3. Older amoeboid stage in division pro-
cess. 4. Beginning of a single fed cell with two nuclei in the
ameboid stage. 5. Well grown schizont containing many nuclei. 6. Schizont's
division and enlargement of each of these into a temporary encystment. 7. Later stage
of encystment. 8. Later stage in encystment, the chromatin mass
are still dividing. 9. Encystment of two cells just before breaking out into the blood;
the internal granules are massed near the outer wall of the encystment. 10. Another
parasite undergoing changes showing encystment in process of formation. 11. Sporo-
cyst containing many very dark oval, and smaller chromatic mass at one end;
chromatic dots are present. 12. Sporocystozoite; note smaller and lighter color,
and larger chromatin mass near center.

PLATE II. — STAGES IN THE LIFE CYCLE OF THE ORGANISM OF TERTIAN MALARIA, *Plasmodium vivax*, IN THE BLOOD OF MAN.

(All drawn under the writer's direction by Miss Ethel Norris from original preparations at a magnification of 2200 diameters.)

1. Trophozoite in ring stage. *2*. Slightly older trophozoite; note ameboid shape, enlargement of red cell and Schüffner's dots. *3*. Older ameboid stage or schizont containing pigment granules. *4*. Infection of a single red cell with two trophozoites in the ameboid stage. *5*. Well grown schizont containing pigment granules; note Schüffner's dots and enlargement of red cell. *6*. Early stage in schizogony (segmentation); the 4 chromatin masses are in division. *7*. Later stage in schizogony; the chromatin masses are still dividing. *8*. Formation of merozoites just before breaking out into the blood; the pigment granules are massed near the center *outside* of the merozoites. *9*. Another parasite undergoing schizogony showing merozoites in process of formation. *10*. Macrogametocyte; note large size, dark color, and smaller chromatin mass at one side; Schüffner's dots are present. *11*. Microgametocyte; note smaller size, lighter color, and larger chromatin mass near center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chromatin mass by successive divisions forms from about 15 to 24 smaller masses (Pl. II, 7, 9). The pigment granules aggregate in the center of the schizont, and then each chromatin mass with a small amount of the surrounding cytoplasm is cut off from the rest of the body (Pl. II, 8). This results in the formation of from 15 to 24 minute cells called merozoites. The merozoites are liberated from the blood cells about 48 hours after infection. With them are also liberated into the blood pigment, the remains of the blood cell, and probably toxins produced by the parasite.

The merozoites when freed from the parasitized cells attach themselves to fresh red corpuscles, become trophozoites and repeat the asexual cycle. Many of the merozoites are probably destroyed by phagocytes or do not succeed in entering fresh cells, but it seems probable that the number of parasitized red cells is increased tenfold at the end of each asexual period of 48 hours.

(4) Period of incubation. The interval between the inoculation of parasites by the mosquito and the appearance of clinical symptoms is called the period of incubation. Clinical symptoms are due to the breaking down of the red cells and the liberation of merozoites, pigment granules, remains of the red cells and probably toxic substances. They do not appear until a sufficiently large number of parasites undergo schizogony at one time — a number estimated by Ross as 150,000,000 in an average man. Ordinarily the period of incubation is 14 to

18 days in tertian malaria, 18 to 21 days in quartan malaria, and 9 to 12 days in estivo-autumnal malaria. These differences are due to the rate of reproduction and probably also to the virulence of the parasite. The tertian parasite undergoes schizogony every 48 hours and each schizont produces from 15 to 24 merozoites, whereas the quartan parasite requires 72 hours for the asexual cycle and each schizont gives rise to only 6 to 12 merozoites; the former, therefore, increases more rapidly and requires a shorter period in which to multiply sufficiently to cause clinical symptoms. The estivo-autumnal parasite undergoes asexual reproduction more quickly than the quartan parasite and is also apparently more virulent, hence the shorter period of incubation. The asexual cycle is repeated again and again in the blood of man and probably may continue for months or years, that is, as long as a person remains infected.

(5) Gametocytes. After several generations of merozoites have been produced, or about a week after infection has taken place, a new stage of the parasite begins to appear in the blood. This is the gametocyte stage. The gametocytes develop from merozoites but no one knows why certain merozoites produce schizonts and others gametocytes. Two kinds of gametocytes are formed, macrogametocytes (Pl. II, 10) and microgametocytes (Pl. II, 11). These can be distinguished in the peripheral blood of man by means of the following characteristics:

	Macrogametocytes	*Microgametocytes*
Size	13–16 microns	9–11 microns
Cytoplasm, stained	darker blue	lighter blue
Chromatin mass	small, eccentric	large central
Melanin	long rods	small rods

(6) Gametogenesis. When subjected to certain stimuli the gametocytes undergo further development. This probably does not occur in the human host but takes place in drawn blood or in the stomach of certain anopheline mosquitoes. After a certain period, probably several weeks, the gametocytes disintegrate in the peripheral blood of man. In drawn blood, however, or in the stomach of the mosquito, they are stimulated, probably by the lowering of the temperature, to form gametes. The nucleus of the macrogametocytes divides and part of the chromatin is cast out (Pl. I, 7b), just as polar bodies are formed by and eliminated from the eggs of higher animals. This process of maturation results in the production of a single, passive macrogamete or female gamete. The nucleus of the microgamete undergoes several successive divisions and then goes through a process called for many years "exflagellation." This is the formation of from 4 to 6 flagella-like bodies, each of which is a nucleated microgamete or male gamete (Pl. I, 7a). As in most higher animals the macrogamete is large and passive and the microgamete small and active. One microgamete fuses with one macrogamete (Pl. I, 8), their cytoplasm becoming mixed together and their chromatin-masses combining to form a single mass. Thus is formed the zygote.

(7) Oökinete. The zygote is at first spherical and passive but soon becomes elongated and active, being capable of gliding movements resembling those of the gregarines. The transformed zygote is now called an oökinete and sometimes a vermicule (Pl. I, 9). No further stages occur in drawn blood but in the stomach of the mosquito the oökinete moves about actively, finally boring its way through the stomach wall and establishing itself between the outer epithelial layer and the muscular layers as a spherical body called the oöcyst (Pl. I, 10).

(8) Oöcyst (Pl. I, 10–12). Fertilization, the change to the oökinete form, and the formation of an oöcyst occupy about 40 hours at ordinary summer temperature. The oöcyst lives at the expense of the surrounding cells, growing enormously in size and eventually projecting like a knob into the body cavity. Infected mosquitoes can be recognized by the presence in the stomach wall of from 1 to 500 of these conspicuous oöcysts.

(9) Sporogony. Inside of the oöcysts the nucleus divides to from a number of daughter nuclei which become the centers of an equal number of cells, the sporoblasts (Pl. I, 11). A residuum of protoplasm containing pigment granules and waste products is left behind. The nucleus of each sporoblast now multiplies by successive divisions; the daughter nuclei migrate to the periphery, and into minute projections that grow out from the sporoblasts. These projections, each with a single nucleus, become spindle-shaped sporozoites and break away

from the remains of the sporoblast (Pl. I, 12). The process of sporogony, that is, the formation of sporozoites within the oöcysts, occupies a period of about 4 or 5 days. The oöcyst bursts shortly after the sporozoites are fully formed and these bodies escape into the body cavity. This cavity in the mosquito differs from the body cavity in higher animals in being a hemocœl containing blood. The blood circulates through this cavity and bathes the various internal organs. With it are carried the sporozoites which may bore their way into various organs but seem to be attracted especially to the salivary glands, where they collect in the secretory cells and in the lumen. The entire cycle in the body of the mosquito from gametocytes to sporozoites occupies a period of about 8 to 12 days according to temperature conditions. The mosquito is now infective and remains so probably for the rest of its life.

(10) Summary. We may summarize the life cycle of the tertian malarial parasite, *Plasmodium vivax*, approximately as follows:

(a) Asexual Cycle in the blood of man.
  a. Period of growth (sporozoites, trophozoites, and schizonts) . . . . . 30 hours
  b. Schizogany (formation of merozoites within the blood cell) . . . . . 18 hours
(b) Sexual Cycle in blood of man and body of mosquito.
  a. Period of growth in blood of man (trophozoites, macrogametocytes and microgametocytes) . . . . . 60(?) hours

b. Oöcyst formation in stomach of mosquito (gametogenesis, fertilization, oökinetes, oöcysts)	40 hours
c. Sporogony within oöcyst in stomach wall of mosquito (formation of sporoblasts and sporozoites)	5 or 6 days

b. *Plasmodium malariæ* (Pl. III, 1–8). Quartan malaria is caused by *Plasmodium malariæ*, the species originally discovered by Laveran in 1880. It is not necessary to give the life cycle of this species in so great detail as that of *P. vivax*, and only the more striking differences between the two species will be noted. These differences are also indicated in the table on page 17.

The period of growth and of schizogony of *P. malariæ* is longer than that of *P. vivax*, i.e., 72 hours instead of 48 hours. The trophozoites are slightly thinner and much less active (Pl. III, 1). The parasitized red cell is no larger than non-parasitized cells but may be darker in color. Pigment is present in the form of large, irregular, slightly motile grains (Pl. III, 4). In stained specimens no degeneration spots (Schüffner's dots) are present in the red cell. The schizonts require about 60 hours to reach their full size; they are very often band-shaped or quadrilateral (Pl. III, 4). Schizogony results in the production of from 6 to 12 merozoites that are arranged in a single ring or rosette (Pl. III, 5–6). The gametocytes are spherical (Pl. III, 7–8).

Double and triple infections with *P. malariæ* sometimes occur. In these cases there are two or three

PLATE III. FIGURES 1–[illegible]. — [illegible] OF [illegible] MALARIA. [illegible] IN THE BLOOD OF MAN.

FIGURES [illegible]. — [illegible] OF THE [illegible].
[illegible]

All drawn under the [illegible] by Miss [illegible] from original preparations at a magnification of 2200 diameters.

P. [illegible]. — [illegible]

P. [illegible]. — [illegible]

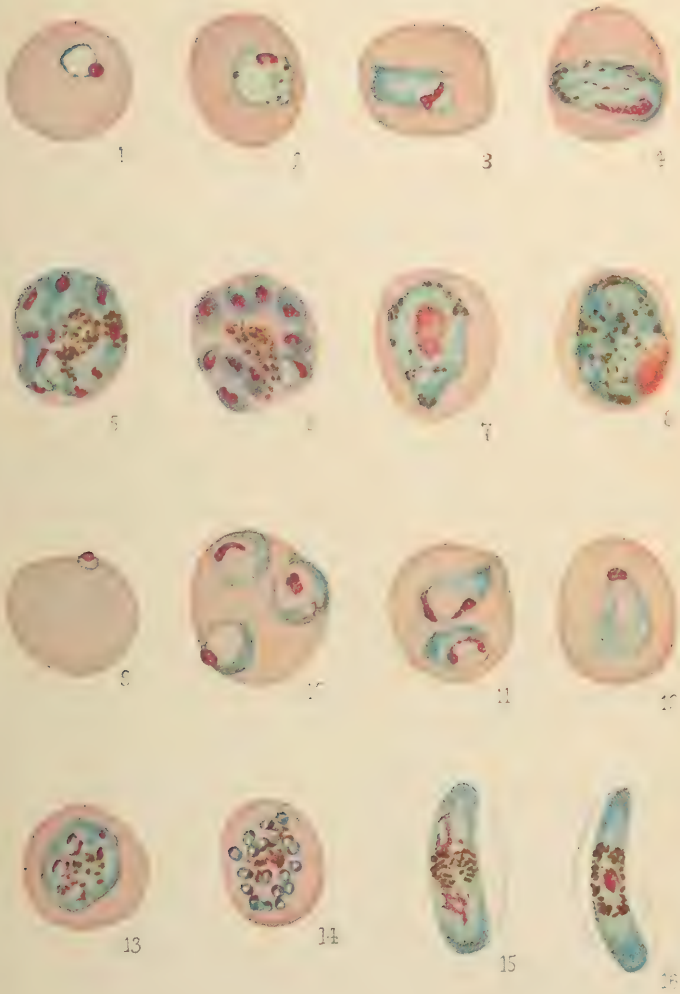
PLATE III, FIGURES *1–8*. — STAGES IN THE LIFE CYCLE OF THE ORGANISM OF QUARTAN MALARIA, *Plasmodium malariæ*, IN THE BLOOD OF MAN.

FIGURES *9–16*. — STAGES IN THE LIFE CYCLE OF THE ORGANISM OF ESTIVO-AUTUMNAL MALARIA, *Plasmodium falciparum*.

All drawn under the writer's direction by Miss Ethel Norris from original preparations at a magnification of 2200 diameters.

P. malariæ. · *1*. Trophozoite in the ring stage *2*. Older trophozoite. *3*. Schizont approximately rectangular in shape; note failure of red cell to become enlarged and absence of Schüffner's dots. *4*. Older schizont. *5*. Presegmentation stage; ten merozoites are being formed; the pigment is aggregated in the center. *6*. Merozoites completely formed. *7*. Microgametocyte; note pale color, and chromatin mass near center. *8*. Macrogametocyte; note darker color and chromatin mass near one side.

P. falciparum. — *9*. Trophozoite in ring stage; note small size, and position at edge, with chromatin dot extended over border. *10*. Triple infection of one red cell with three young schizonts. *11*. Double infection of one cell with older schizonts. *12*. Old schizont just before disappearance from peripheral blood; note small size and failure of red cell to become enlarged. *13*. Presegmentation stage; note chromatin in separate masses and pigment aggregated near center. *14*. Merozoites fully formed; note small size, large number, and irregular distribution of merozoites. *15*. Microgametocyte; note crescent shape, comparative thickness, blunt ends, wide distribution of chromatin, and pigment granules. *16*. Macrogametocyte; note comparative thinness, end not so blunt and chromatin in center surrounded by wreath of pigment granules.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groups of parasites that undergo the asexual cycle independently and at intervals of about 24 hours. This is due to inoculations by mosquitoes on different days. Clinical symptoms appear in cases of single infection every 72 hours, in cases of double infection on two successive days and then again on two successive days after an interval of 48 hours, and in cases of triple infection every 24 hours. Mixed infections with *P. malariæ* and *P. vivax* may occur at the same time in a single patient.

c. *Plasmodium falciparum* (Pl. III, 9–16). Estivo-autumnal, tropical, quotidian, subtertian, or malignant tertian malaria is caused by *P. falciparum*. This species exhibits the following characteristics. The asexual cycle is not so definite in length as that of the other two species requiring from 24 to 48 hours. Schizogony very rarely occurs in the peripheral blood. The trophozite is small and actively ameboid (Pl. III, 9). One red cell is often infected by two parasites and less often by three or more (Pl. III, 10–11); and when a severe infection occurs there may be more parasites present than red cells. The schizont is small, being only about 5 microns in diameter (Pl. III, 12). It undergoes division in the capillaries of the internal organs giving rise to 8 to 30 or more merozoites (Pl. III, 13–14) which may be arranged in a rosette or irregularly. The parasitized red cells are not enlarged; they may be partly decolorized, and sometimes large, reddish degeneration spots, called Maurer's dots, may be present.

The gametocytes are so different from those of the other two species that this type has by some been referred to a separate genus and called *Laverania malariæ*. Both macrogametocytes and microgametocytes are crescentic in shape. The macrogametocytes (Pl. III, 16), are slender; stain a deeper blue; and possess a single mass of chromatin in the center surrounded by a compact circle of pigment. The microgametocytes (Pl. III, 15) are broader; stain a lighter blue; and possess chromatin and pigment granules scattered irregularly throughout the middle third of the crescent. During maturation in the stomach of the mosquito the macrogametocyte becomes ovoid and then spherical; the microgametocyte likewise becomes ovoid and then spherical and then the microgametes are produced as in the other two species.

Infections with *P. falciparum* and *P. vivax* or with *P. falciparum* and *P. malariæ* or with all three may occur in a patient at the same time. These mixed infections are more difficult to diagnose than infections with only one species of parasite and one should not make a report as soon as one type has been found but should continue his examination until he is convinced that he is or is not dealing with a mixed infection.

3. *Methods of Making Films for the Diagnosis of Malaria.* 1. Thin films. a. Equipment: Clean glass slides; small bottle of alcohol; small package absorbent cotton; Hagedorn needle fastened in cork of small vial and extending down into alcohol.

DISTINGUISHING FEATURES OF THE THREE SPECIES OF PLASMODIUM OCCURRING IN MAN

	P. vivax	*P. malariæ*	*P. falciparum*
1. Type of fever	Tertian (benign tertian) simple or double	Quartan; simple, double or triple	Estivo-autumnal; quotidian; subtertian; malignant tertian
2. Length of asexual cycle	48 hours	72 hours	24–48 hours
3. Stages in peripheral blood	All	All	No segmenting stages
4. Parasites in infected red cells	Usually one in each	Usually one in each	Often two or more in one cell
5. Movement of young trophozoite	Active ameboid	Very slow ameboid	Active ameboid
6. Size of infected red cells	Larger than normal	About normal	About normal
7. Color of infected red cell	Nearly normal	Darker than normal	Partially decolorized
8. Granules in infected red cells	Schüffner's dots	None	Maurer's dots
9. Pigment	Short rods, actively motile	Grains, large, irregular, very slightly motile	Few, small, irregular grains, feebly motile
10. Shape of schizont	Circular	Quadrilateral	Circular
11. Number of merozoites	15–24	6–12	8–10 or more
12. Arrangement of merozoites	Irregular or 2 rings	One ring, a rosette	Irregular or rosette
13. Gametocytes	Spherical or ovoid	Spherical or ovoid	Crescentic

b. Obtaining blood: Clean ear lobe or end of finger with alcohol. Puncture with needle. One drop of blood is mounted one half inch from the end of the slide. Place the end of another slide near the drop of blood at an angle of 30 degrees to 45 degrees with the shorter end of the slide. Draw this slide along until it touches the drop. When the blood has spread along the edge push the slide fairly rapidly toward the other end. A thin film will result, covering about one half of the slide. Allow the film to dry, then write data directly in it with a lead pencil.

c. Fixing and staining. (1) Wright's, Leishman's, or Hasting's stain: Cover film with a few drops of the stain and allow to remain 1 minute. Add double the volume of distilled water. After 5 minutes, wash, and dry in air. The cytoplasm of the parasite stains blue and the chromatin red. The pigment remains brown, unstained.

(2) Giemsa's stain: Fix in absolute methyl alcohol for 5 minutes, wash gently. Stain in one part Giemsa plus ten parts distilled water for ten minutes; or in one drop of Giemsa per cc. of water over night. Wash. Dry. The results are similar to those obtained by the use of Wright's or Leishman's stains.

2. Thick films. a. Equipment: Same as for thin films.

b. Obtaining blood: Same as for thin films, except several drops are obtained near center of slide and spread with the needle over an area of one half to three fourths of a square inch. Allow to dry.

c. Fixing and staining: Fix and decolorize in 95 per cent alcohol plus 2 per cent HCl for one half hour. Wash in running tap water a few minutes. Stain as with thin films. In these preparations more blood cells are present per unit area and hence the presence of parasites is more easily determined.

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B. Organisms that Cause Trypanosomiasis (Sleeping Sickness and Chagas' Disease) in Man

1. *Classification.* These diseases are caused by blood-inhabiting Protozoa known as trypanosomes. They belong to the class Mastigophora, whose members are characterized by the presence of one or more permanent whip-like locomotor organs called flagella. The flagellates comprise both free-living and parasitic species. For the sake of convenience the flagellates that spend part of their life cycle in the blood

of vertebrates and the other part in the digestive tract of a blood-sucking invertebrate are usually termed Hemoflagellates. To this group belong the trypanosomes and leishmanias.

2. *Description of Species.* Among the more important species of trypanosomes that are pathogenic in lower animals are *T. brucei*, the organism of nagana in mammals, *T. evansi*, the organism of surra in cattle, camels, etc., *T. equiperdum*, the organism of dourine in horses, *T. equinum*, the organism of "mal de caderas" in horses and mules, and *T. hippicum*, the organism of murrina in mules. The number of species of trypanosomes that are pathogenic in man is not certain; those usually recognized are *T. gambiense*, *T. rhodesiense*, and *T. cruzi*.

a. *T. gambiense* (Pl. IV, D, 1–3). This species was named by Dutton in 1902 from specimens taken from a fever patient in tropical Africa. It is the cause of one type of sleeping sickness. It ranges from 18 to 30 microns in length and 1.5 to 2.5 microns in width. Polymorphism is exhibited by this species, there being short, stumpy forms 14 to 20 microns long, intermediate forms 20 to 24 microns long, and long forms 23 to 33 microns long. Tsetse flies of the species *Glossina palpalis* transmit it from man to man.

b. *T. rhodesiense.* This species is so similar to *T. gambiense* in morphology that the two are difficult to separate. *T. rhodesiense* occurs in northwestern Rhodesia, and Portuguese and German East Africa, and is transmitted by *Glossina morsitans.* This is

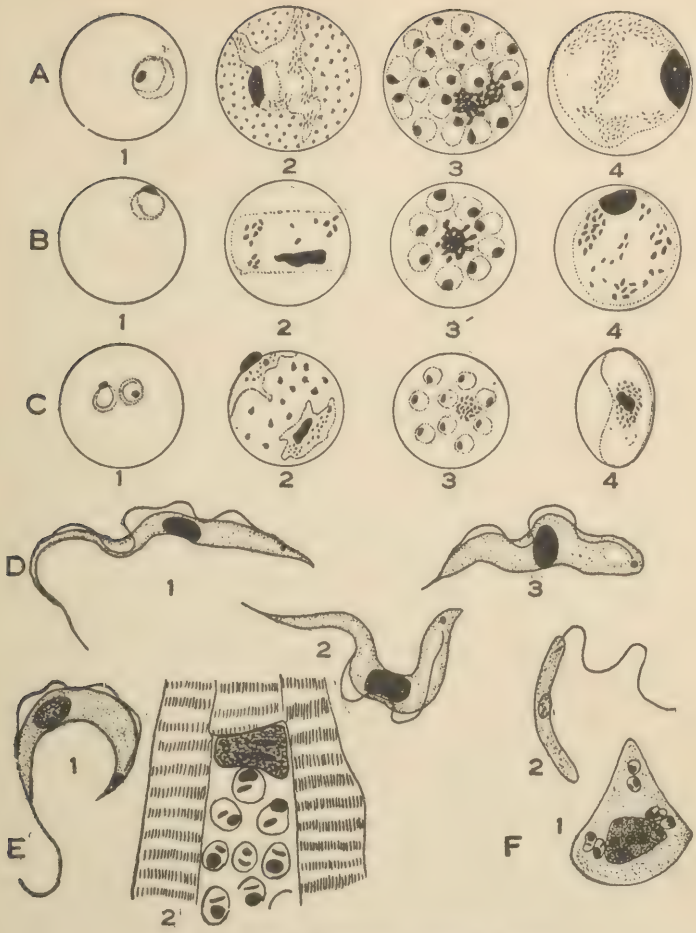

PLATE IV. — BLOOD INHABITING PROTOZOA OF MAN.

(The three top rows, *A*, *B*, and *C*, show 4 similar stages in the life cycle of the malarial organisms in the blood of man.)

Row *A* — *Plasmodium vivax* of tertian malaria; Row *B* — *P. malariæ* of quartan malaria; and Row *C* — *P. falciparum* of estivo-autumnal malaria. No. *1* in each row represents a red cell containing trophozoites in the ring stage; No. *2*, schizonts; No. *3*, merozoite formation; and No. *4*, gametocytes.

Fig. *D*, *1* to *3*. *Trypanosoma gambiense* (× 1400). *1*. Long form. *2*. Intermediate form. *3*. Short form. (After Castellani and Chalmers.)

Fig. *E*, *1* and *2*. *Trypanosoma cruzi* (× 1400). *1*. Trypanosome stage in the blood. *2*. Leishmania stage in muscle. (From photographs by W. H. Taliaferro.)

Fig. *F*, *1* and *2*. *Leishmania* (× 1400). *1*. Tissue stage of *L. donovani*. (After Brumpt.) *2*. Flagellate stage *L. tropica*. (After Row.)

a much more virulent organism than *T. gambiense* and more difficult to influence therapeutically.

c. *T. cruzi* (Pl. IV, E, 1–2) causes Chagas' disease in Brazil. It is about 20 microns long and passes through a leishmania stage, especially in the muscles (E, 2). Multiplication by division does not occur in the blood, but in the muscles during the leishmania stage. The bug, *Triatoma*, is the transmitting agent.

3. *Methods of Diagnosis.* Blood films should be made and examined fresh, or stained as described for the malarial parasites (page 18). The trypanosome nature of the organism can be recognized at once, but the species diagnosis is very difficult. Organisms are usually so few in the blood that ordinary blood smears are useless for purposes of diagnosis. Several other methods have been found to be of value, the examination of centrifuged blood and material from gland puncture being the best. After the sleeping sickness stage has set in trypanosomes may be found in the spinal fluid.

(1) Secure 10 to 20 c.c. of citrated blood, centrifuge and examine the leucocyte layer.

(2) Gland puncture with a dry, sterile hypodermic needle.

(3) Inoculate guinea-pigs with blood or gland juice and examine their blood from time to time.

Trypanosomes are present in the blood in Chagas' disease only in the acute stage. Diagnosis in the chronic stage must be made from history and clinical symptoms.

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C. Organisms that Cause Leishmaniosis in Man

1. *Classification.* The organisms that cause leishmaniosis are included among the Hemoflagellates, and belong to the genus *Leishmania.* Forms with flagella usually appear only in cultures.

2. *Description of Species.* Kala-azar is caused by *Leishmania donovani;* the so-called infantile kala-azar is probably caused by the same organism; oriental sore is due to the presence of *L. tropica;* and espundia or American leishmaniosis is caused by *L. americana.*

a. *L. donovani* (Pl. IV, F, 1), occurs in many parts of India, China, Southern Russia, and in certain lands bordering on the Mediterranean. It is found only occasionally in the blood, but usually lives within the cells of the spleen, liver, lymph glands and endothelial cells of the blood and lymph vessels. The organisms are spherical or oval in shape and

vary from 2 to 4 microns in diameter. Each contains a large nucleus and a small rod-shaped body. Multiplication occurs in the invaded cells, and the parasites, when they break out, are often devoured by leucocytes. When cultivated outside of the body flagellated stages appear. The bed bug has been suspected as the transmitting agent.

b. *L. tropica* (Pl. IV, F, 2) is the causative agent of oriental sore in India, Persia, Syria, Arabia and Northern Africa. The organisms are present outside and within the cells of the sore. They are often spindle-shaped and about 3 microns long.

c. *L. americana* causes espundia in certain regions of tropical South America. Skin sores are produced by the attacking organisms.

3. *Methods of Diagnosis.* The principal methods of diagnosing kala-azar are (1) the examination of blood smears, (2) study of material from spleen and liver punctures, and (3) the culture of blood or splenic juice in N. N. N. medium. Sterling's gentian violet stain is of particular value in staining smears from tissues. This is prepared and used as follows: Grind 5 grams of gentian violet in a mortar with 10 cc. of 95 per cent alcohol. Add 2 cc. of aniline oil and then 88 cc. of distilled water. Continue to grind for a short time; allow to stand several days; and filter. Cover the dried smear with the stain and after about ten seconds rinse in tap water.

Splenic puncture is described by Manson as fol-

lows (pp. 156–157): "*Splenic puncture* must not be lightly undertaken. A preliminary examination of the blood should always be made, not only with a view to ascertain the presence of the Leishman body, but to exclude leucocythæmia and obviate the necessity for splenic puncture, and the attendant risk of fatal hæmorrhage so easily induced in that disease. Death from hæmorrhage may follow this seemingly trivial procedure. When the liver is enlarged, being a less vascular organ and less easily torn, it should be selected for puncture in preference to the spleen. The abdomen had better be fixed firmly with a binder to prevent as far as possible movement of the diaphragm and consequent risk of tearing the punctured organ. A fine hypodermic needle, scrupulously clean and dry, and connected with the barrel of the syringe by a short length of rubber tubing, should be used, the patient being directed not to start or breathe while the puncture is being made. Failure to draw blood is not to be regarded as failure to obtain material for microscopical examination; on the contrary, it is an advantage, as the object is to procure spleen or liver pulp, not blood. After blowing out the contents of the needle on a slip, a film should be spread, dried and stained."

Special Literature on Leishmaniosis

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3. Intestinal Protozoa of Man

A. Methods of Fecal Diagnosis of Intestinal Protozoa

1. *Obtaining Material.* Feces should be collected in a dry, clean container. The presence of urine or antiseptics tends to kill the protozoa; and water hastens the multiplication of coprozoic species, and hence makes the diagnosis of entozoic forms more difficult. Formed stools usually contain cysts and these may be recognizable even after several weeks. Samples from different parts of the stool should be taken for examination, since cysts may be irregularly distributed through the fecal mass. Trophozoites may occur in formed stools, but are more frequently encountered in liquid stools. The length of time after the stool is passed before active protozoa begin to degenerate varies for the different species. It is best, however, in any case to examine material as soon as possible — within an hour or two if conditions are favorable — since diagnosis becomes more difficult with time. Intestinal protozoa do not, as a rule, encyst after leaving the body, hence there is no object in keeping liquid stools in the hope of obtaining cysts. Active specimens may be obtained from patients passing formed stools by the use of purgatives. Of these saline is the best, since castor oil and others result in the presence of oil droplets in the stool which are confusing. It is the general impression among protozoölogists that more reliable results are obtained without the use of purgatives. In cases of suspected amebic dysentery some of the

bloody mucus should be examined, since *Endamœba histolytica* is often more numerous in this material than in other parts of the stool. Frequently charcot-leyden crystals are present in the feces of patients suffering from amebic dysentery; their presence is not a certain criterion, however, since they also occur in infections with parasitic worms. A vacuum bottle serves admirably as a container in which to collect material. It may be rinsed with water heated to 37° and a bottle containing the fecal sample suspended in it. For shipping purposes, tin salve boxes or paper containers may be used. Cysts may be kept for years if the stool containing them is emulsified in 10 per cent formalin. Amebic cysts preserved in this way lose their glycogen and hence when stained with iodin exhibit a vacuole instead of a brown mass.

2. *Methods of Examination.*

a. *Fresh smears.* Take a very small amount of fresh fecal material on the end of a toothpick and rub it up on a glass slide in a drop of 0.7 per cent saline solution. The resulting smear, when covered with a cover glass, should be thin enough so that print can easily be seen through it. Such a preparation is especially useful for the recognition of active endamœbæ and flagellates and often of cysts, and avoids the incorrect diagnosis of body cells as endamœbæ, as is often done with stained material. After considerable practice the presence of protozoa can be recognized with the lower power (16 mm.) objective, but because of their minute size it is

usually necessary to use the high dry objective (4 mm.) to determine species.

b. *Staining with iodin.* The best solution for this purpose is probably 5 per cent potassium iodid in normal salt solution saturated with iodin. Fecal material should be stirred up on a glass slide in a drop of this mixture and examined under a cover glass. Nuclei, glycogen masses, and other structures are stained in this solution and hence more distinctly seen than in fresh smears. Fresh mixtures should be made each week.

c. *Donaldson's iodin-eosin smear method (as modified by Kofoid, Kornhauser and Swezy).* This method differs from that just described by the addition of a saturated solution of eosin in normal salt solution. The solution should be made up fresh every day by taking two parts of the eosin mixture, one of the iodin, and adding two parts of normal salt solution. The eosin in this mixture provides a pink background and stains the bacteria, fecal débris, etc., immediately, whereas the protozoan cysts are stained yellow and glycogen masses within them brown.

d. *Eosin or neutral red.* These two dyes may be used to determine whether cysts are living or dead. When fecal material is stirrred up in one part of dye to 10,000 parts of water, dead cysts and fecal débris, etc., stain at once, but the living cysts remain unstained, appearing as bright, refractile spheres.

e. *Multiple examinations.* Intestinal protozoa are more or less irregularly distributed in the stool and

are usually only intermittently present in the stools of infected persons. To obtain accurate results it is, therefore, necessary to examine material from different parts of the stool and from stools passed on different days. The per cent of positives obtained by this procedure has been found in practice to be much greater than when only one examination is made. The most practical procedure is to study three smears.

f. *Concentration methods.* Several methods have been devised of treating stools so as to concentrate the protozoan cysts contained in them and thus make the diagnosis more certain. These methods are too long and complicated to be used in the field and are necessary in the laboratory only under special conditions. They also frequently result in the distortion of the cysts and hence render species diagnosis difficult. The method of Cropper and Row as modified by Boeck is as follows: "Take at least one gram of the stool to be examined, place it with thirty cubic centimeters of normal saline solution in the mixing glass and stir for at least ten minutes with an electric mixer such as is used at soda fountains in mixing drinks. At the end of ten minutes, while still stirring, add five cubic centimeters of ether and stir two or three minutes longer. Pour the emulsion into a separatory funnel and allow to stand for at least five to seven minutes, during which the cysts will settle to the bottom in the saline solution and débris will float in the ether above. The funnel used for this separation has a funnel-shaped bowl

with steep sides contracting to a narrow neck above the turncock.

"At the end of this period of standing, the saline solution, about fifteen cubic centimeters, is drawn off at the bottom of the separatory funnel into a centrifuge tube of a capacity of fifteen cubic centimeters, and is centrifuged for three minutes at 1,600 revolutions per minute. The supernatant fluid is then drawn off and the residue is examined microscopically for the cysts. At this time a drop of neutral red is applied to a small amount of this residue to procure a sharper contrast between the cysts and the surrounding débris. By this method a fecal examination can be completed in twenty-five to thirty minutes."

g. *Schaudinn's alcoholic sublimate iron-hematoxylin method.* In case it is impossible to identify the organisms by the methods described above, this procedure may be followed to bring out the details of nucleus, flagella, etc. (a) Prepare a fixing solution as follows: Saturated solution of mercuric chloride in distilled water, 200 cc.; 95 per cent alcohol, 100 cc.; glacial acetic acid, 15 cc. Heat to 65° C.

(b) Make a smear on a slide and while still wet drop it into the warm fixing solution. Leave there for about ten minutes.

(c) Immerse in 70 per cent alcohol, containing a trace of iodin 30 minutes to 24 hours; wash in water a few minutes; immerse in 3.5 to 4 per cent aqueous solution of iron alum, 1 to 4 hours; wash well in

water; transfer to 0.5 per cent aqueous solution of hematoxylin, 4 to 24 hours.

(d) Differentiate in 1.75 to 2 per cent iron alum solution until enough of the stain has been removed. This is best done by examining under the microscope at frequent intervals; wash well in large amount of water; pass up through alcohols to absolute; transfer to xylol; mount in balsam.

(e) Eosin may be used in the absolute alcohol if a counter stain is desired.

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B. Intestinal Amœbæ of Man

1. *Classification.* The amœbæ belong to the class Sarcodina. The members of this class are characterized by the presence of locomotor organs in the form of temporary finger-like projections of protoplasm called pseudopodia. Many free-living species are common in fresh water; comparatively few species are parasitic. Of these *Endamœba histolytica* is pathogenic and a very important cause of dysentery, especially in tropical and subtropical countries.

2. *Species.* There are six well-established species

of amœbæ living in man. These are *Endamœba histolytica*, *E. coli*, *Endolimax nana*, *Iodamœba williamsi*, and *Dientamœba fragilis*, which live in the intestine, and *Endamœba gingivalis* that occurs in the mouth and has been accused, probably unjustly, of causing pyorrhea alveolaris.

3. *Endamœba histolytica.*

a. *Distribution.* This is apparently the only pathogenic human amœba. It has been found wherever examinations for it have been made and probably exists wherever there are human beings. Many surveys that have been made, especially since the beginning of the late war, to determine the distribution of intestinal protozoa in various countries, have shown that infections with this species, as well as with other species, are not so restricted to the tropics as was formerly generally supposed, but occur almost as frequently in the colder regions of the globe. A résumé of the results of over 35 surveys published between 1916 and 1919 (Hegner and Payne, 1921) shows that on the average about 9 per cent of all persons are infected with *E. histolytica.* Most of these did not exhibit clinical symptoms but were "carriers." In the carriers the amœbæ live on the tissues of the intestinal wall, forming small ulcers, but repairs are made by the body before clinical symptoms are evident. Symptoms appear when the ulcers become large. Only a small percentage of infected persons ever show symptoms; apparently the number is greater in the tropics than in the temperate zone. This species is disseminated

in the cyst stage, and since carriers pass cysts every carrier is potentially dangerous. Patients while they are suffering from diarrhea or dysentery are of no importance in transferring the disease, since the amœbæ are swept out of their intestine before they have an opportunity to encyst and are not infective in the motile stage. When the stools of dysenteric patients become formed again, cysts reappear, resulting in "convalescent carriers." Infections once acquired seem to last indefinitely if not treated.

b. *Trophozoite* (Pl. V, 1).

(1) Living. The trophozoite of *E. histolytica*, when rounded, ranges from 18 to 40 microns in diameter, but is usually 20 to 30 microns. It may be distinguished in the living condition from the trophozoites of free-living amœbæ by the absence of contractile vacuoles, and from those of other intestinal amœbæ by the method of pseudopodial formation, character of the food, and appearance of the nucleus. Specimens of *E. histolytica* that have recently been passed progress in a slug-like manner and rather rapidly, and very little difference can be detected between the ectoplasm and endoplasm. Soon active locomotion ceases and thin ectoplasmic pseudopodia are sent out in an explosive fashion — a characteristic that does not occur in *E. coli*. *E. histolytica* differs from other intestinal amœbæ in its food habits. It ingests red blood cells, less often leucocytes and tissue elements, but seldom bacteria, although these may invade the organism. Other intestinal amœbæ do not eat red blood cells

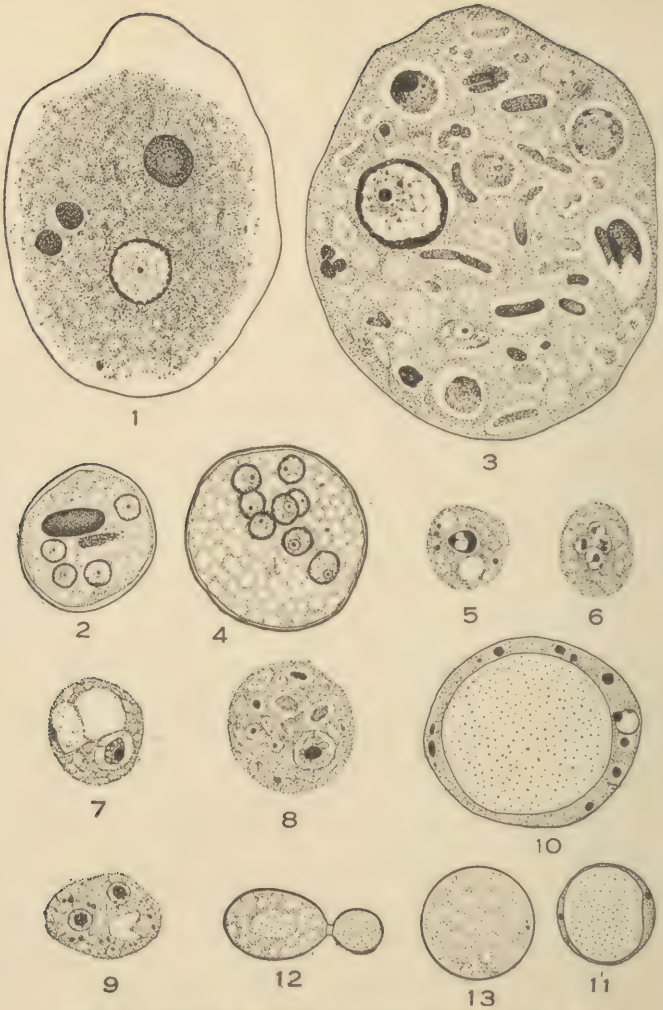

PLATE V. — INTESTINAL AMŒBÆ AND VEGETABLE ORGANISMS OF MAN.
(All magnified about 1500 diameters.)

1. Endamœba histolytica, motile vegetative stage. *2. E. histolytica*, cyst. *3. Endamœba coli*, motive vegetative stage. *4. E. coli*, cyst. *5. Endolimax nana*, motile vegetative stage. *6. Endolimax nana*, cyst. *7. Iodamœba williamsi*, cyst. *8. I. williamsi*, motile vegetative stage. *9. Dientamœba fragilis*, motile vegetative stage. *10–11. Blastocystis hominis*, large and small stages. *12.* Intestinal yeast, budding. *13.* Intestinal mold. (Figs. *1–5*, *9*, after Dobell; Figs. *6–8*, after Taliaferro and Becker; Figs. *10–11*, original; Figs. *12–13*, after Kofoid, Kornhauser and Swezy.)

but feed on bacteria. A third character of the living trophozoite of *E. histolytica* of diagnostic value is the nucleus, which is sometimes faintly visible but usually invisible in contrast to that of *E. coli* which is comparatively conspicuous.

(2) Stained trophozoite. The trophozoite must be stained in order to bring out the characteristics of the nucleus, which is very important in diagnosis. The best staining method for this purpose seems to be the Schaudinn iron-hematoxylin method described above. The nucleus is spherical and vesicular, and usually measures from 4 to 7 microns in diameter. It has a distinct membrane, on the inside of which is a thin peripheral layer of chromatin. In the center of the nucleus is a small karyosome surrounded by an achromatinic capsule. No chromatin is located between the karyosome and the periphery of the nucleus. The chief difference between the nucleus of *E. histolytica* and *E. coli* is the small amount of chromatin in the former and the large amount in the latter. Another difference is the central position of the karyosome in *E. histolytica* and the eccentric position of the karyosome in *E. coli.*

c. *Precystic stages.* The trophozoites of *E. histolytica* live and multiply by binary division in ulcers in the walls of the large intestine. Many of them move out into the lumen and become precystic. They decrease in size, lose all their food inclusions, become sluggish and round up. It is best not to attempt to diagnose specimens in this condition, since at this time the nucleus often contains a thick

peripheral layer of chromatin and an eccentric karyosome, thus resembling *E. coli*.

d. *Cysts* (Pl. V, 2). These range from 5 to 20 microns in diameter and possess a wall that is thin, transparent, smooth and without spines. The nucleus resembles that of the trophozoite. As the cyst passes down the intestine the nucleus divides into two and these divide again to form four. Supernucleated cysts containing 8 nuclei may occur. A glycogen vacuole is present in the young cysts but disappears later, probably being used up as food material. This stains dark mahogany in iodin but is dissolved out when fixed and appears in stained specimens as a vacuole. Chromatoid bodies also occur in the cysts. At first there may be several of these, but as the cyst becomes older they are absorbed. The shape of the chromatoid body is characteristic, being rod-like with rounded ends.

For a comparison of *E. histolytica* with other intestinal amœbæ, see the table on page 41.

4. *Endamœba coli.*

a. *Distribution.* *E. coli* has also been recorded from every region of the earth where surveys of intestinal protozoa have been made. The incidence of infection is even greater than that with *E. histolytica*, being about 20 per cent.

b. *Trophozoite* (Pl. V, 3).

(1) Living. The living trophozoite of *E. coli* measures from 18 to 40 microns in diameter, being usually from 20 to 30 microns. The size, therefore, is of no value in distinguishing this species from

E. histolytica. There is no marked difference between ectoplasm and endoplasm and the pseudopodia are never thin and hyaline as in *E. histolytica*, and are not protruded explosively as in the latter. The endoplasm is more granular and usually well filled with food vacuoles containing almost everything present in the intestine except red blood cells and tissue elements. The nucleus is clearly visible in living specimens, except when obscured by food, and the heavy layer of chromatin granules around the periphery is distinct.

(2) Stained trophozoites. The nucleus is the most important structure in *E. coli* for diagnostic purposes. It measures from 4 to 8 microns in diameter and when stained with iron-hematoxylin is seen to differ from that of *E. histolytica* in the possession of (*a*) a peripheral layer of large chromatin granules, closely packed and tending to become thicker on one side (*b*) a much larger karysome, eccentrically placed, with a more pronounced achromatinic capsule around it, and (*c*) chromatin granules between the karyosome and nuclear membrane.

c. *Precystic stages*. As in *E. histolytica* the trophozoite becomes smaller before encystment, throws out all food material, and becomes spherical in shape. It is almost impossible to distinguish it from precystic stages of *E. histolytica*.

d. *Cysts* (Pl. V, 4). The cysts of *E. coli* range from 10 to 30 microns in diameter. Cysts that are less than 10 microns in diameter are usually of

E. histolytica and those greater than 20 microns of *E. coli*. The mature cysts possess 8 nuclei and are usually without chromatoid bodies; when these are present they are never rod-like with rounded ends as in *E. histolytica*, but resemble particles of splintered glass. The glycogen mass is well defined and usually larger than it is in *E. histolytica*.

5. *Endamœba gingivalis*. This was the first of the amœbæ living in man to be described (Gros, 1849). It lives in the human mouth in dental caries and between and about the base of the teeth. In diameter it ranges from 7 to 60 microns, the usual size being 10 to 20 microns. Locomotion in this species resembles that of *E. coli* but is more rapid. The endoplasm, which is fairly well differentiated from the ectoplasm, contains bacteria, tissue elements, etc. Cysts of this species are unknown and probably do not exist, transmission from one host to another taking place directly from mouth to mouth.

6. *Endolimax nana* (Pl. V, 5–6). This species was not discovered in man until 1908 and was not distinguished from other species until 1916. The trophozoite (Pl. V, 5) ranges from 6 to 12 microns in diameter and is characterized by the presence of several blunt pseudopodia at different parts of the body at the same time. It moves very little. Within the endoplasm may be seen food vacuoles containing bacteria and various particles of intestinal débris but never blood cells or tissue elements. The nucleus measures from 1 to 3 microns

in diameter and is principally characterized by the presence of one, two or more large masses of chromatin connected by strands. This peculiar distribution of the chromatin led to the placing of this species in a separate genus, *Endolimax*.

Cysts (Pl. V, 6). The cysts of *Endolimax nana* are usually oval in shape, and measure 8 to 10 microns by 7 to 8 microns. When mature there are four nuclei which correspond in structure to those of the trophozoites. Glycogen masses are sometimes seen in young cysts but almost never in the four-nucleated cysts. No chromatoid bodies are present at any stage.

7. *Iodamœba williamsi* (Pl. V, 7–8). This intestinal amœba has become well known only within the past few years, its cysts having been designated "iodin cysts" or "I-cysts." Species resembling *I. williamsi* have been recorded from the pig and monkey, and some of these may be the same as that in man. Very little is known about its incidence in human beings but it is probably rather common. It resembles *E. coli* in its movements, and the character of its food inclusions, but is smaller, measuring from 5 to 20 microns in diameter; usually 9 to 13 microns. The nucleus, which is 2 to 3 microns in diameter, is distinctive, with all of the chromatin massed together as a large central karyosome around which are a series of granules. The cysts (Pl. V, 7) are more or less spherical but often irregular in shape, 8 to 12 microns in diameter, and uninucleate. A very conspicuous characteristic is the presence of

a large glycogen mass which persists in old cysts for as long as several weeks after they are passed. This is denser and sharper in outline than the glycogen masses in the cysts of other species. In the nucleus of the cyst the karyosome moves to one side and the nuclear granules to the other, giving it a very characteristic appearance.

8. *Dientamœba fragilis* (Pl. V, 9). This species was described and named in 1918 and has been seen by only a few observers. It disintegrates soon after being passed, which probably accounts for its apparent rarity. It is very small, ranging from 3.5 to 12 microns in diameter — usually 5 to 6 microns. The ectoplasm and endoplasm are sharply separated, and the wave-like active movement of the pseudopodia is characteristic. Most specimens contain two nuclei which have their chromatin in a centrally located karyosome which consists of several spherical granules embedded in a more lightly staining matrix. Cysts of this species are unknown.

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DISTINGUISHING FEATURES OF THE CYSTS OF FOUR SPECIES OF INTESTINAL AMŒBÆ OF MAN

Characteristic	*Endamœba histolytica*	*Endamœba coli*	*Endolimax nana*	*Iodamœba williamsi*
Stained with iodin *				
Glycogen	Present in young cysts; absent in older cysts; stains dark mahogany	Well defined; usually larger than in *E. histolytica*	Sometimes in young cysts	Large, dense, sharply defined; persists in old cysts
Stained with iron-hematoxylin				
Diameter	5 μ–20 μ	10 μ–30 μ	8 μ–10 μ x 7 μ–8 μ	8 μ–12 μ
Shape	Spheroidal	Spheroidal	Ellipsoidal	Spheroidal or irregular
Nuclei, number	1–4; when mature	Usually 8	4 when mature	One
Nuclei, structure	About 1/6 diam. of cyst in 4-nucleate cysts; about 1/3 diam. in 1-nucleate cyst; karyosome small, centrally located; thin peripheral layer of chromatin	From 1/5 to 1/6 diam. of cyst in 8-nucleate cysts; about 1/3 diam. in 1 nucleate cyst; karyosome large, eccentric; heavy peripheral layer of chromatin	1–1.3 μ in 4 nucleate cysts; one, two or several large masses of chromatin connected by strands; no peripheral layer of chromatin	2.5–4 μ in diam.; one large karyosome on one side, nuclear granules on opposite side
Chromatoid bodies	Usually one or none in mature cysts; rod-like, with rounded ends	When present in pieces resembling splintered glass	None	None

*The character of the nuclei of the various species can be seen more or less distinctly when stained with iodin. Usually the chromatin masses are distinguishable, enabling one, with practice, to determine whether the specimen belongs to the genus *Endamœba*, *Endolimax*, or *Iodamœba*.

DISTINGUISHING FEATURES OF MOTILE STAGES OF FOUR SPECIES OF INTESTINAL AMŒBÆ OF MAN

Characteristic	*Endamœba histolytica*	*Endamœba coli*	*Endolimax nana*	*Iodamœba williamsi*
Living, unstained				
Size	18 μ–40 μ, usually 10 μ–30 μ	18 μ–40 μ, usually 20 μ–30 μ	6 μ–12 μ	5 μ–20 μ, usually 9 μ–13 μ
Locomotion	When very fresh, locomotion slug-like and rapid, difference between ectoplasm and endoplasm slight, very soon progressive locomotion ceases and thin, hyaline blade-like pseudopodia are explosively extended, with a sharp demarcation between ectoplasm and endoplasm	Slow; pseudopodia formed by flowing out slowly; with practically no difference between ectoplasm and endoplasm	Very little, several blunt pseudopods at one time; distinction between ectoplasm and endoplasm varies	As in *E. coli*
Nucleus	Faint or invisible	Clearly visible unless obscured by food	Faintly visible	Visibility depends on amount of food inclusions
Food	Tissue elements, leucocytes, red-blood cells. No bacteria	Bacteria and débris, but no red-blood cells or tissue elements	Bacteria and débris, but no red-blood cells or tissue elements	Bacteria and débris; but no red-blood cells or tissue elements
Stained with hematoxylin				
Nucleus	4 μ–7 μ in diam.; karyosome small, centrally located; thin peripheral layer of chromatin	4 μ–8 μ in diam.; karyosome large, eccentrically located; heavy peripheral layer of chromatin	1 μ–3 μ in diam.; one, two or several large masses of chromatin connected with strands; no peripheral layer of chromatin	2 μ–3 μ in diam.; one large central karyosome surrounded by a layer of refractile granules

C. Intestinal Flagellates of Man

1. *Classification.* The intestinal flagellates of man belong to the class Mastigophora and are characterized by the presence of one or more flagella. There is great need of careful investigation of the intestinal flagellates, since only a few are known at all well, and many species have been described and named but are not yet well established. The best known forms are *Giardia lamblia*, *Trichomonas hominis*, and *Chilomastix mesnili.* These occur so frequently that they require more extended treatment than those less well known. Among the latter are *Enteromonas hominis* and *Embadomonas intestinalis.*

2. *Description of Species.* a. *Giardia lamblia* (Pl. VI, 6–7). Motile stage: When in the motile stage this species is pear-shaped, from 10 to 21 microns long and from 5 to 12 microns wide. The anterior half of the organism bears a depression which acts as a sucking disk for attachment to intestinal epithelial cells. Two nuclei, two axostyles, and four pairs of flagella are present as shown in the figure. Specimens in the motile stage are not so frequently observed in feces as are the cysts. Rats, mice, rabbits, and other common animals are often infected with what are probably distinct species of giardias and those who wish to gain a preliminary knowledge of these organisms should examine material from the duodenum of these animals.

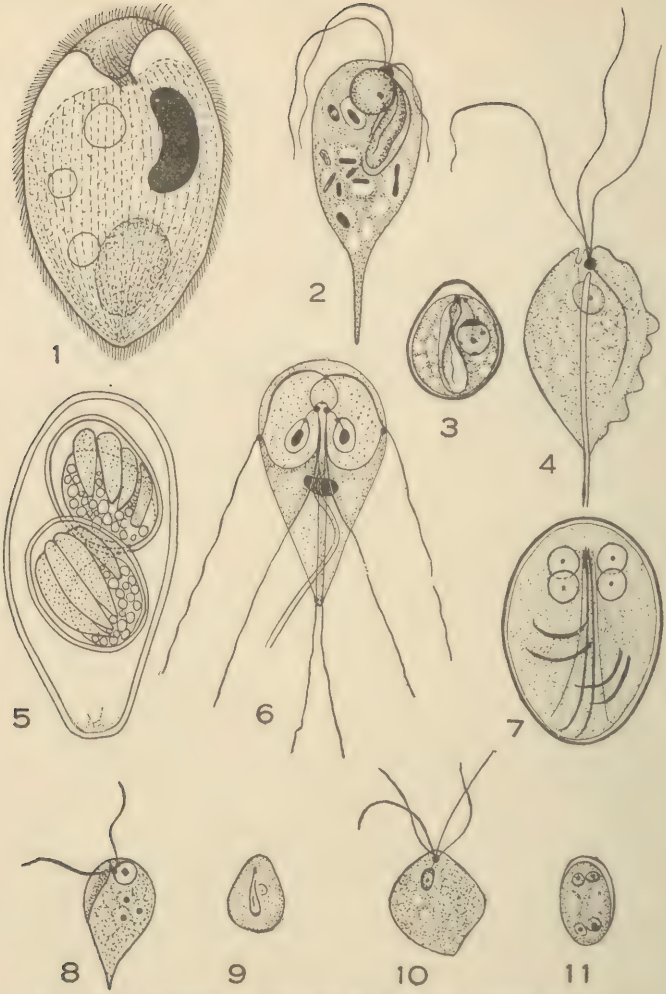

PLATE VI. — INTESTINAL FLAGELLATES, CILIATES AND COCCIDIA OF MAN.

(Fig. *1*, magnified 585 diameters; Fig. *5*, magnified 1775 diameters; all other figures magnified about 2300 diameters).

1. Balantidium coli. 2. Chilomastix mesnili, motile trophozoite. *3. Chilomastix mesnili*, cyst. *4. Trichomonas hominis*, motile trophozoite. *5. Isospora hominis*, oöcyst with two sporoblasts, each of which contains 4 sporozoites. *6. Giardia lamblia*, motile trophozoite. *7. Giardia lamblia*, cyst. *8. Embadomonas hominis*, motile trophozoite. *9. Embadomonas hominis*, cyst. *10. Enteromonas hominis*, motile trophozoite. *11. Enteromonas hominis*, cyst. Although these figures (except Figs. *1* and *5*) are all drawn to the same scale and hence indicate comparative sizes, it should be remembered that considerable variation exists in the size of the members of each species. (*1*, after Leuckart; *2*, after Boeck; *3* and *7*, after Kofoid, Kornhauser and Swezy; *4*, after Faust; *5* and 11, after Dobell; *6*, after Simon; *8* and *9*, after Broughton-Alcock and Thomson; *10*, after Chalmers and Pekkola.)

Cysts (Pl. VI, 7): These are oval bodies 8 to 15 microns long and 6 to 10 microns wide. Two or four nuclei are present, usually at one end, and two longitudinal curved axostyles extend down the center of the cyst. Two rod-shaped parabasal bodies and a variable number of loops which probably represent the cytostomal fibrils are also embedded in the cytoplasm.

Surveys of intestinal protozoa show that *Giardia lamblia* is very widely distributed, having been found wherever examinations have been made, and that about 12 per cent of the general population is infected.

b. *Trichomonas hominis* (Pl. VI, 4). Motile stage: This is a pear-shaped organism measuring from 10 to 15 microns long and 3 to 4 microns wide. An axostyle is situated near the center of the body and projects beyond the posterior end. Along one side is an undulating membrane terminating at the posterior end in a flagellum. Three other flagella extend out from the anterior end. The cytoplasm is vacuolated. Within it, near the anterior end, are a nucleus, containing scattered chromatin granules, and a parabasal rod. Reproduction is by binary fission. No cysts have yet been identified with certainty. Specimens of *T. augusta*, which resemble the species found in man, are abundant in the intestine of the frog, and *T. muris* may be obtained from the cecum of the rat.

Trichomonas hominis is geographically as widespread among human beings as is *Giardia lamblia*

but the incidence of infection is lower, namely about 3 per cent.

c. *Chilomastix mesnili* (Pl. VI, 2–3). Motile stage: This may also be described as a pear-shaped organism, rounded anteriorly and pointed posteriorly. It varies considerably in size, ranging from 7 to 18 microns in length. Three flagella extend out freely from the anterior end, and a fourth flagellum lies within the cytostome. The cytostome is about one-half the length of the body. A large spherical or oval nucleus lies near the anterior end.

Cysts (Pl. VI, 3): These are usually pyriform but often spherical and measure 6 to 9 microns in diameter. A single nucleus is present containing a chromatin granule near the center and chromatin masses on the membrane. Extending across the cyst are the remains of the cytostome characteristic of this species.

Chilomastix mesnili and *Trichomonas hominis* were not recognized as separate species until 1910. Before this time both species were called by protozoölogists *Trichomonas*, and by others they were usually known as *cercomonads*. No species of cercomonad, however, inhibits the human intestine. Recent studies indicate that *Chilomastix mesnili* is cosmopolitan in its distribution and that about 4 per cent of all persons are infected with it — a higher percentage than with *Trichomonas hominis*.

d. *Embadomonas intestinalis* (Pl. VI, 8–9). This intestinal flagellate has recently been reported from various countries, including Egypt, England, and the

United States. The motile form is ovoid in shape, 5 to 6 microns in length and 3 to 4 microns in breadth, with a nucleus near the anterior end, two blepharoplasts near by from which spring two flagella, one thin, the other thicker, and a large, elongated mouth on one side near the anterior end.

The cysts (Pl. VI, 9) are somewhat pear-shaped, 4.5 microns to 6 microns long and about 3 microns broad. They are difficult to stain satisfactorily but a single nucleus and a long curved fibril are visible within.

The incidence of infection with this species among human beings is probably very low.

e. *Enteromonas hominis* (Pl. VI, 10–11). A number of flagellates to which different names have been given probably belong to this species. They have been reported from Brazil, Egypt, India, etc., but are probably not commonly present in the human intestine. The motile stage is oval or rounded, 4 to 8 microns in diameter, with a vesicular nucleus, two or more small blepharoplasts, and four anterior flagella, three of which are free and extend forward; the fourth may project backward and is more or less adherent to the surface of the body.

The cysts (Pl. VI, 11) are oval, 6 to 8 microns long and 3 to 4 microns broad, with four nuclei when mature.

3. *Methods of Diagnosis.* (1) Living specimens. The motile forms of intestinal flagellates may be seen moving about in fecal smears if material is examined shortly after being passed by the patient.

Giardia lamblia moves about very slowly and makes very little progress. When it turns on its side the convexity of the dorsal surface and concavity of the ventral sucking disk are obvious. Its shape when at rest resembles that shown in Plate VI, Fig. 6; at this time the pair of ventral flagella are actively in motion. The living cysts of *G. lamblia* are oval in shape. Within them can be seen some of the fibrils that are more clearly brought out by stains.

Trichomonas hominis. Living specimens of *Trichomonas* may be distinguished from *Chilomastix* by the presence of an undulating membrane, the wave-like movements of which can be seen beginning at the anterior end and progressing posteriorly. The axostyle may also be seen projecting from the posterior end of *Trichomonas*, but care should be observed not to mistake the spine-like extension at the posterior end of *Chilomastix* for the axostyle of *Trichomonas*. No cysts of *Trichomonas hominis* are known.

Chilomastix mesnili. This species appears larger than *Trichomonas* and broader toward the anterior end. It has no undulating membrane. The cysts are pear-shaped.

(2) Stained specimens. Specimens of motile stages and cysts of the intestinal flagellates when fixed in Schaudinn's solution and stained with iron-hematoxylin show the structures indicated in Plate VI.

(3) Cultural methods. *Trichomonas hominis*, *Chilomastix mesnili*, and *Embadomonas intestinalis* have been grown in artificial cultures. Work recently

carried on in this laboratory indicates that a greater percentage of positive cases may be obtained from cultures than from the examination of smears. The medium as prepared by Hogue (1921) consists of a hen's egg shaken up in a flask with glass beads; to this is added 200 cc. of Locke's solution; this is heated over a water bath in motion for 15 minutes, then filtered through cotton, tubed, and autoclaved. A tooth pick with a small portion of the fecal sample on one end is dropped into a tube and incubated at 37° C. If the above named flagellates were present in the motile stage they should appear in the culture medium on the second or third day. *Embadomonas* will encyst in cultures (Hogue).

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D. Intestinal Coccidia of Man

1. *Classification.* The Coccidia are Sporozoa of the subclass Telosporidia. They are as a rule parasitic in epithelial cells of vertebrates and invertebrates, and reproduce by both schizogony and sporogony. Among the best known Coccidia are *Coccidium schubergi* of the centipede, *Eimeria stiedæ* of the rabbit, and *E. avium* in birds. Many other species are known, but most of them not in detail.

2. *Description of Species.* Prior to the year 1915 only ten cases of coccidiosis in man had been reported and these were supposed to be due to the same parasites as those found in rabbits, cats and dogs. Recently many more cases have been discovered and it seems probable that the human coccidia are more numerous than heretofore suspected.

a. *Isospora hominis* (Pl. VI, 5). This species was first described by Virchow in 1860. Wenyon and others have recently reported many cases of infection of soldiers suffering from dysentery and enteritis and invalided to England from Gallipoli. This species has also been recorded from men who had been in Egypt, Saloniki, and Mesopotamia.

The oöcysts in the feces are elongate, ovoid in form, 25 to 33 microns in length, and 12.5 to 16 microns in width. They are usually unsegmented

when passed in the stools. Two sporoblasts are formed in each oöcyst and each sporoblast produces four vermiform sporozoites.

b. *Eimeria wenyoni* is a rare species discovered by Wenyon in 1915 and four cases have been recorded. The oöcyst is spherical, about 20 microns in diameter and with an outer rough surface. Within the oöcyst are four sporoblasts each containing two sporozoites. These are already differentiated when the oöcysts are passed by the patient.

c. *Eimeria oxyspora* is also rare. The oöcyst is spherical and about 36 microns in diameter. Within it are four sporoblasts, each with two sporozoites.

d. *Eimeria snijdersi* was recently described from Sumatra. The oöcyst is large, and spherical, and about 45 microns in diameter. Four sporoblasts are formed, each with two sporozoites.

3. *Methods of Diagnosis.* The oöcysts of coccidia appear when the feces are treated as described on page 27. Perhaps the best way to become acquainted with them is to examine the feces of rabbits, which are very highly infected. Freshly passed oöcysts of the rabbit coccidium, *Eimeria stiedae*, are almost filled with protoplasm. If the feces are mixed with water the oöcysts will develop and within about 48 hours four sporoblasts will form within them, each of which will be seen to contain two sporozoites.

Special Literature on Coccidia

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E. Intestinal Ciliates of Man

1. *Classification.* The ciliates belong to the class Infusoria. The members of this class are characterized by the presence of locomotor organs in the form of cilia. Most of them are free living. Many parasitic species occur in vertebrates and invertebrates, but only one species, *Balantidium coli*, has been found with frequency in man. Other species have been recorded from man but not often enough to warrant their inclusion here.

2. *Description of Species.* a. *Balantidium coli* (Pl. VI, 1). This is a very large Protozoön, measuring from 50 to 100 microns in length and from 40 to 70 microns in breadth. It is oval in shape and covered with cilia arranged in parallel rows, giving it a striated appearance. The macronucleus is large and bean-shaped and near it lies a small spherical micronucleus. At the anterior end is a funnel-shaped peristome, and at the posterior end a terminal cytopyge (anus). Two contractile vacuoles are present. Reproduction is by binary fission. Conjugation and encystment occur.

3. *Method of Diagnosis.* There is nothing in fecal material that is likely to be confused with these ciliates on account of their large size and distinctive characteristics. A species that may be *Balantidium coli* is abundant in the intestine of the pig. Anyone wishing to study these parasites can easily obtain them from this animal.

F. Some Vegetable Organisms in Human Feces

There are many bodies that occur in human feces that may be mistaken for the cysts of Protozoa. Of these the most confusing are probably the vegetable organisms known as *Blastocystis hominis*, and the yeasts.

1. *Blastocystis hominis* (Pl. V, 10–11). This organism is frequently found in stools containing intestinal Protozoa and often occurs when Protozoa are absent. It is usually spheroidal in shape and very variable in size, ranging from 3 to 20 microns in diameter. The smaller specimens are often oval.

2. *Intestinal Yeasts* (Pl. V, 12–13). Certain yeasts are normally present in human feces and may be mistaken for protozoan cysts. In eosin stain they take on a red color at once which is sufficient to distinguish them from protozoan cysts. Some of them also are found in the process of budding. Other cyst-like bodies also occur in human feces; these may be degenerating organisms, or the spores of molds (Pl. V, 13).

G. Coprozoic Protozoa

Coprozoic protozoa are nonparasitic species that may occur in fecal material. Representatives of the Rhizopoda (amœbæ), Mastigophora (flagellates) and Infusoria (ciliates) are included among the coprozoic forms. It is important to realize that this type of Protozoon exists since many students have mistaken species that are present in the feces by chance, for parasitic forms. Coprozoic species may be taken into the human body in the cyst stage with food or drink, pass through the alimentary canal unharmed, and multiply in the deposited feces. They may also gain access to the fecal material after it is passed. The best account of this subject will be found in Chapter IX of Dobell and O'Connor's "Intestinal Protozoa of Man." We shall here simply mention briefly certain types that may be encountered during fecal examinations and to warn the diagnostician against confusing them with true parasites.

1. *Amœbæ.* "The commonest coprozoic Rhizopods found in human feces are the small amœbæ commonly, but incorrectly, called '*limax* amœbæ,' or amœbæ of the *limax* group." There are several ways in which these forms may be distinguished from the parasitic species. (1) Large numbers of active amœbæ present in a stale stool are probably coprozoic forms, since parasitic species become quiescent soon after leaving the body. (2) Coprozoic amœbæ usually possess a single contractile vacuole, a structure that

is absent from parasitic species. (3) The nuclear structure of coprozoic forms differs from that of parasitic species, hence any specimens that exhibit nuclear characteristics differing from those described for the parastic species are probably coprozoic. These characteristics can, of course, only be made out in specimens properly stained with iron-hematoxylin. Coprozoic forms can be cultivated easily on simple mediums, whereas parasitic species cannot. A useful medium is that of Walker and Sellards (1913): agar, 2.50 gm.; sodium chloride, 0.05 gm.; Liebig's beef extract, 0.05 gm.; normal sodium hydroxide solution, 2.00 cc.; distilled water, 100.00 cc.; sterilize in autoclave.

2. *Flagellates.* Among the most common coprozoic flagellates are species of the genera *Cercomonas* and *Bodo*. *Cercomonas longicauda* has often been referred to as a parasitic species, since its discovery in human feces by Wenyon in 1910. Many of the so-called *new* intestinal flagellates that are from time to time described from man probably are coprozoic species.

3. *Ciliates.* A number of species of ciliates have been described from human feces and have been considered by many students as human intestinal forms although they were probably present because of contamination after defecation. These include *Chilodon dentatus* (Guiart, 1903), *Chilodon uncinatus* (Manson and Sambon, 1909), *Colpoda cucullus* (Schulz, 1899) and *Uronema caudatum* (Martini, 1910).

PART II

WORMS PARASITIC IN MAN

PART II

WORMS PARASITIC IN MAN

BY

William W. Cort

1. Introduction

The endoparasitic helminths belong to the classes Trematoda and Cestoda under the phylum Platyhelminthes and to the class Nematoda under the phylum Nemathelminthes. The trematodes, commonly known as flukes, are important parasites of man, especially in the Far East and Africa, where they produce such dangerous diseases as bilharziasis, Japanese schistosomiasis, clonorchiasis and paragonimiasis. The cestodes or tapeworms are practically cosmopolitan in distribution. While they are frequently encountered, their relation to disease is not so definite except in the case of Echinococcus, which in man produces hydatids of the liver and other organs. The nematodes or roundworms are the most prevalent and important helminths of man. In this group belong the organisms which produce hookworm disease, filariasis and trichinosis. It is only since medicine and public health work have come to be considered as world problems that the diseases produced by parasitic worms have come

into prominence. Since such diseases are very prevalent in the Tropics and Orient and there is constant danger of their spread into new regions with commerce and immigration, it is important that the medical man, wherever located, should be acquainted with their manifestations and methods of diagnosis, and should be able to identify the worms which produce them and know their methods of entrance into man.

In the discussion which follows the attempt will be made to bring together in clear, compact form that information which a physician or field worker would need in diagnosing by laboratory methods the diseases of man produced by parasitic worms. There will also be included certain methods which can be utilized in the preservation or investigation of the various stages in the life cycles of these organisms. The reader is referred to the textbooks of Parasitology and Tropical Medicine in the bibliography at the beginning of this book for detailed information on the biology of the organisms or for medical information in regard to the diseases which they produce.

2. Knowledge of Geographical Distribution as an Aid to Diagnosis

A. General Discussion

Very often a knowledge of the distribution and incidence of the parasitic worms of man may be of great aid in diagnosis. There is therefore included

here a table which gives data on these points. Certain factors related to distribution are of interest in this connection. The commonest and most widely distributed of human worms are those which have no intermediate host or free development, but infect a new host with eggs which escape with the feces of the old. In certain of these forms the egg is immediately infective, as in *Hymenolepis nana* and *Enterobius* (*Oxyuris*) *vermicularis* and in others there is necessary a period of larval development within the egg outside the body as in *Trichuris trichiura* and *Ascaris lumbricoides*. Other widely distributed forms depend for their spread on a food habit as in the case of *Diphyllobothrium latum*, the infective stage of which is found in the muscles of fish, *Tænia solium* and *Tænia saginata* which depend on the eating of raw or partly cooked pork and beef respectively and *Trichinella spiralis* which depends on pork eating. In the case of Echinococcus man gets the infestation from eggs from the feces of the dog, which gets it from the larval stages in the pig or sheep. Therefore this parasite is most prevalent in those regions where man, the dog and sheep are closely associated. Those nematodes which have a free stage in the soil as the hookworm and *Strongyloides stercoralis* are limited to tropical and subtropical regions where the conditions of temperature and moisture are favorable for the development of the free-living stage. The members of the family Filaridæ are transmitted to man by the bite of blood-sucking insects and are therefore limited in

distribution by the distribution of effective intermediate hosts. Certain parasites, as the sheep liver fluke, are rare in man but common in domesticated animals. These forms must be considered whenever unusual eggs are found in fecal examinations because, since in many cases they are cosmopolitan in distribution in their true hosts, they may appear in man in any region. There are also a number of other forms the true status of which is doubtful, which may either prove to be incidental parasites or fairly common when the regions where they are found are more completely investigated. The distribution of the trematodes offers problems of particular interest, since they must have as intermediate hosts certain specific molluscs. Their distribution is therefore limited by the distribution of effective intermediate hosts as well as by their method of entrance into man. The infective stage of the schistosomes penetrates actively through the skin of man. In certain other important trematodes human infestation is acquired by eating the flesh of animals containing the infective cysts, in the case of the human lung fluke from crabs, and in the human liver fluke from fish. In the following table of the distribution of the parasitic worms of man some of the rarest or doubtful forms are omitted.

B. Table of Distribution and Incidence of Worms Parasitic in Man

1. *Trematodes*

Scientific Name	Common Name	Organ Inhabited	Incidental or Rare in Man*	Geographical Distribution
Gastrodiscoides hominis		Large intestine	*	India, Cochin China
Watsonius watsoni		Small intestine	*	Africa
Echinostoma ilocanum		Intestine	*	Philippine Islands
Euparyphium malayanum		Intestine	*	India, Malaya
Echinochasmus perfoliatus var. japonicus		Intestine	*	Japan
Fasciolopsis buski	Large intestinal fluke	Small intestine		China, Indo-China, Formosa, Sumatra, India
Heterophyes heterophyes		Small intestine	*	Egypt
Heterophyes nocens		Small intestine	*	Japan
Metagonimus yokogawai	Small intestinal fluke	Small intestine		Japan, Korea, Formosa, China
Fasciola hepatica	Sheep liver fluke	Bile ducts	*	Cosmopolitan in sheep, etc.
Clonorchis sinensis	Human liver fluke	Bile ducts		Japan, China, Korea, Formosa, Cochin China
Opisthorchis felineus		Bile ducts	*	Europe, Siberia, Japan
Opisthorchis noverca		Bile ducts	*	India
Opisthorchis viverrini		Bile ducts	*	Siam
Paragonimus westermani	Human lung fluke	Lungs		Japan, Korea, Formosa, Philippine Islands, Peru
Schistosoma haematobium	Human blood fluke	Portal veins		Africa, Near East, Portugal
Schistosoma mansoni	Human blood fluke	Portal veins		Africa, West Indies, Northern South America
Schistosoma japonicum	Human blood fluke	Portal veins		Japan, China, Philippine Islands

* Those forms which are incidental or rare in man are marked with a star in this column.

TABLE OF DISTRIBUTION AND INCIDENCE OF WORMS PARASITIC IN MAN

2. *Cestodes*

SCIENTIFIC NAME	COMMON NAME	ORGAN INHABITED	INCIDENTAL OR RARE IN MAN*	GEOGRAPHICAL DISTRIBUTION
Diphyllobothrium latum	Human broad or fish tapeworm	Small intestine		Europe along coast of Baltic, North Sea, Swiss and Italian Lakes, Japan, Africa, and North America
Diphyllobothrium cordatum		Intestine	*	Greenland and Iceland
Diplogonoporus grandis		Intestine	*	Japan
Sparganum mansoni		Connective tissues	*	Japan, China, Africa, British Guiana, etc.
Sparganum proliferum		Connective tissues	*	Japan and United States
Dipylidium caninum		Small intestine	*	Cosmopolitan in dog and cat
Hymenolepis nana	Dwarf tapeworm	Small intestine		Cosmopolitan
Hymenolepis diminuta		Small intestine	*	Cosmopolitan in rat
Davainea madagascariensis		Intestine	*	Madagascar, Mauritius, Siam, British Guiana
Davainea formosana		Intestine	*	Formosa
Tænia solium	Human pork tapeworm	Intestine		Cosmopolitan
Tænia saginata	Human beef tapeworm	Intestine		Comsopolitan
Tænia confusa		Intestine	*	United States
Echinococcus granulosus	Hydatid cysts	Liver and other organs		Cosmopolitan but especially in Iceland, Australia and Argentine
Echinococcus multilocularis		Liver and other organs		Germany, Italy, Switzerland, Russia, Siberia

* Those forms which are incidental or rare in man are marked with a star in this column.

TABLE OF DISTRIBUTION AND INCIDENCE OF WORMS PARASITIC IN MAN

3. Nematodes

SCIENTIFIC NAME	COMMON NAME	ORGAN INHABITED	INCIDENTAL OR RARE IN MAN *	GEOGRAPHICAL DISTRIBUTION
Rhabditis hominis		Intestine	*	Japan
Strongyloides stercoralis		Small intestine		In tropical and sub-tropical countries
Ancylostoma duodenale	Hookworm	Small intestine		In tropical and sub-tropical countries
Ancylostoma ceylonicum		Small intestine	*	India, Siam, Malaya
Necator americanus	Hookworm	Small intestine		In tropical and sub-tropical countries
Trichostrongylus orientalis		Intestine		Japan
Ternidens diminutus		Large intestine	*	Africa
Œsophagostomum apiostomum		On walls of caecum and colon	*	Africa
Eustrongylus visceralis		Kidney	*	Cosmopolitan in dogs
Gnathostomum spinigerum		Subcutaneous tissues	*	Siam
Physaloptera mordens		Small intestine	*	Africa
Trichinella spiralis	Trichina	Small intestine and muscles		Wherever pork is an important article of human food
Trichuris trichiura	Human whip worm	Large intestine		Cosmopolitan
Ascaris lumbricoides	Human round worm	Small intestine		Cosmopolitan
Lagochilascaris minor		Intestine	*	Trinidad, B. W. I.

* Those forms which are incidental or rare in man are marked with a star in this column.

TABLE OF DISTRIBUTION AND INCIDENCE OF WORMS PARASITIC IN MAN — *Continued*

3. Nematodes — Continued

SCIENTIFIC NAME	COMMON NAME	ORGAN INHABITED	INCIDENTAL OR RARE IN MAN*	GEOGRAPHICAL DISTRIBUTION
Enterobius (Oxyuris) vermicularis	Human pin or seat worm	Large intestine		Cosmopolitan
Dracunculus medinensis	Guinea worm	Subcutaneous connective tissue		Africa, Arabia, and Near East, India
Filaria bancrofti	Human filaria	Lymphatics and blood stream		Wide tropical and subtropical distribution
Filaria ozzardi		Lymphatics and blood stream	*	West Indies and Northern South America
Loa loa	Eye worm	Connective tissue and blood stream		Tropical West Africa
Acanthocheilonema perstans		Connective tissue and blood stream		Tropical Africa
Dirofilaria magalhaesi		Heart and blood vessels	*	Brazil
Onchocerca volvulus		Subcutaneous connective tissue		West Coast of Africa
Onchocerca cæcutiens		Subcutaneous connective tissue of head		Guatemala

* Those forms which are incidental or rare in man are marked with a star in this column.

3. Clinical Symptoms as an Aid in Diagnosis

Often the clinical manifestations of the diseases produced by parasitic worms are an aid in diagnosis, especially when coupled with a knowledge of geographical distribution. In most cases, however, the clinical diagnosis will need to be confirmed by a laboratory examination, since the symptoms produced by parasitic worms rarely produce clear cut clinical pictures. In a region where paragonimiasis is known to exist pulmonary symptoms with a rusty expectoration would always suggest a sputum examination for the eggs of *Paragonimus westermani* or in Egypt, haematuria would always suggest the presence of *Schistosoma hæmatobium*. Certain obscure intestinal disturbances may suggest the need of fecal examination to determine whether intestinal worms are present, and anal itching will suggest an examination for *Enterobius* (*Oxyuris*) *vermicularis*. It is only in trichinosis and echinococcosis that the physician must depend chiefly on clinical manifestations for his diagnosis. The reader is referred to a textbook of medicine for a discussion of the symptomatology of these two diseases.

4. Diagnosis by Blood Examination

A. Eosinophilia. There very commonly develops in cases of infestation with parasitic worms a definite increase in the number of eosinophil cells. The discovery of a high degree of eosinophilia is of great significance in the diagnosis of trichinosis, and any

increase above normal of these cells in the blood should suggest the need of a fecal examination for the eggs of parasitic worms.

B. Identification of Microfilariæ in Blood. Since the larval stages of the Filaridæ are found in the blood stream, examinations of the blood are used in the diagnosis of these forms. It should be remembered that the embryos of *Filaria bancrofti*, the most important of these forms, are only found in the peripheral blood at night. When microfilariæ are present in large numbers in the blood stream, they may be easily recognized in a wet film preparation, but usually it is necessary to fix and stain them. The following account of the preparation of blood films for the demonstration of microfilariæ is taken from Manson's "Tropical Diseases." (See bibliography.)

"A large-sized drop (20 c. mm.) should be taken and spread so as to occupy an area of half a square inch on the slide. For this purpose the finger should be pricked with a broad-pointed needle and the under surface dabbed with four good-sized drops. The film is allowed to dry, protected from dust (especially cotton fibers, which may simulate microfilariæ), dehemoglobinized, and stained at the same time in a dilute watery solution of fuchsin (5 drops to 150 cc. distilled water), then examined in a wet state under a low power of the microscope.

"If permanent hematoxylin preparations are required, after dehemoglobinization, films must be fixed in alcohol and ether (equal parts), and stained

with Delafield's hematoxylin for about 7 minutes. They are then differentiated momentarily in acid alcohol, washed in tap-water till blue, dried, and mounted in Canada balsam. For *Filaria bancrofti* of the periodic variety the blood should be taken after 10 P.M., and for *Loa loa* after 10 A.M."

There are so many doubtful microfilariæ reported in the literature that it is a difficult matter to decide which should be considered as distinct species. A knowledge of the geographical distribution of these forms (see table above) is a considerable aid to diagnosis. The following key, which is taken from Stitt (see bibliography) and figures of the more important microfilariæ are included as an aid in diagnosis.

Key to the Better-known Species of Microfilariæ in the Blood of Man

I. Sheath present.

a. No periodicity.

Filaria philippinensis. Tightly fitting sheath, not flattened out beyond extremities; tail pointed and abruptly attenuated; 290 μ to 320 μ by 5 μ.

b. Periodicity.

1. Nocturnal periodicity.

Filaria bancrofti (Pl. VIII, 3). Tail pointed; sheath loose; V-spot 90 μ from head; break in cells 50 μ from head; 300 μ by 7.5 μ

2. Diurnal periodicity.

Loa loa (*Filaria loa*). (Pl. VIII, 2). Tail pointed; sheath loose, V-spot 60 μ to 70 μ from head; break in cells 40 μ from head; 245 μ by 7 μ.

II. Sheath absent; no periodicity.
   a. Tail blunt.
      Acanthocheilonema perstans (*Filaria perstans*) (Pl. VIII, 4). 190 μ to 200 μ by 4.5 μ to 5 μ.
   b. Tail sharply pointed.
      1. *Filaria ozzardi* (*F. demarquayi*) (Pl. VIII, 1). 200 μ by 5 μ.
      2. *Onchocerca volvulus*. Microfilariae of this species have not been seen in blood but only in lymph spaces around females. 250 μ to 300 μ by 5 μ to 7 μ.

5. Methods of Fecal Examination

A. *General Discussion.* The final diagnosis in the majority of infestations with parasitic worms must rest on whether the eggs or larvæ can be found in the feces. In the diagnosis of *Paragonimus westermani* an examination of the sputum is necessary, and in *Schistosoma haematobium* the eggs are found in the urine, which can be sedimented or centrifugated for examination. In most cases it is possible to make the determinations rapidly and accurately.

The technique used is of very great importance. The number of worms present varies greatly in the different cases and even with the best possible technique it is probable that not all cases will be detected. Since in the lighter infestations there are usually no symptoms present, such individuals are usually classed as "carriers." Since with almost all the parasitic worms of man an injurious effect is produced in light infestations as well as heavy, although there may be no noticeable symptoms, and

since every "carrier" is a potential spreader of the disease, it would seem that the greatest possible accuracy in diagnosis is desirable. There are, however, other factors to be considered. Greater accuracy usually means fewer examinations. When, as in hookworm campaigns, the examinations to be made are many and the workers few, a compromise must be made between thoroughness and speed. Under hospital conditions where there are only a comparatively few examinations to be made, it would seem to be advisable to use the slower but more critical methods. In field campaigns or surveys, however, where thousands of examinations must be made, often by microscopists who have no special scientific training, and under conditions where it is difficult to obtain or use complicated apparatus, simple, more rapid methods of examination would certainly be preferable. Descriptions will be given here of the smear method, the centrifugation method, the Kofoid-Barber brine-loop-flotation method, and the Willis-Molloy brine flotation method.

B. *Smear Method.* The smear is the most direct and simple method of fecal examination. In preparing a smear a small bit of the feces to be examined is mixed with distilled or filtered water on an ordinary glass microscopical slide. The smear should be mixed in enough water so that ordinary print can be seen through it. If the smears are too dense it greatly lessens the chances of finding the eggs. The accuracy of the smear method depends on the number of slides examined. The examination of a single

smear will instantly detect heavy infestations, but to detect lighter cases would require the examination of such a large number of slides that the method loses its value.

C. *Centrifugation Method.* The object of centrifugation is to wash and concentrate the fecal material to be examined. Various modifications of this method have been used. All have as common features (1) the thorough mixing of a piece of fecal material with distilled or filtered water, (2) the straining of this mixture through a sieve or piece of cheesecloth to remove larger particles, (3) the centrifugation of the suspension to concentrate the material, and (4) the making of smears of the residue for examination. Usually the sample should be washed and centrifugated about three times. The time of centrifugation should be very short because the eggs are easily thrown down. The simplest application of the centrifugation method is that used at the Immigration Hospital at Angel Island, California, for examinations of oriental immigrants. Here the sample used is about the size of the thumb, and the sediment after centrifugation is spread over the whole surface of a 1 x 3 slide and is examined without a cover glass. In the hookworm campaigns of the Rockefeller International Health Board in the West Indies a combination of the smear and centrifugation methods is used. Two or three smears are made from each sample to eliminate the heaviest cases and the negatives are examined after centrifugation.

D. *The Kofoid-Barber Brine-loop-flotation Method.* The mixing of fecal samples with some solution heavier than the parasitic eggs, to concentrate them at the surface of the mixture, has long been used. A special modification of this method was devised by Kofoid and Barber for the examination for hookworm of men in the Army from the Southern States during the war. In this method a fecal sample is thoroughly mixed with concentrated brine. The coarse float is forced below the surface with a disk of No. 0 steel wool and the container allowed to stand for about an hour for the eggs to ascend. The surface film is then looped off onto a slide and examined without a cover glass. The microscope must be focused on the surface of the drop on the slide. This method gives fine concentration and a very clear preparation for study.

E. *The Willis-Molloy Brine-flotation Method.* Two field directors of the International Health Board, Drs. Willis and Molloy, have independently developed modifications of the brine flotation method which they have found useful in making large numbers of fecal examinations under tropical conditions. In their method the one-fourth or preferably one-half ounce capsule containers which are used for collecting the fecal samples are also used for mixing with the salt solution, so that there is no necessity of changing the feces into a new container. Boards to which lids of these same containers are tacked are used as holders for the sample containers during the process of examinations. These boards can be

arranged for whatever number of containers seems most convenient. After the opened containers containing the fecal samples are placed in the holders, the sample of feces in each one is thoroughly mixed with concentrated salt solution and filled up to the brim. A 2 x 3 inch glass slide is then placed over the container in contact with the surface of the salt solution. This is left for one half hour to one hour to give the eggs a chance to come to the top. The slide is then carefully removed and examined under the microscope. Reports from the workers who have used this method in the field and experiments in our own laboratories indicate that a greater concentration of eggs is obtained by this method than when the surface film of the flotation mixture is looped off onto a slide.

F. *Limitations of Flotation Methods:* It is sometimes well to use the flotation method in combination with a preliminary single smear to eliminate the heaviest positives, but with such a simple technique as the Willis-Molloy method this is hardly necessary. Unfortunately operculate eggs do not float in salt solution, so that the flotation method cannot be used where it is desirable to detect such forms as the human liver fluke, *Clonorchis sinensis* or the fish tapeworm, *Diphyllobothrium latum*. Also it is of no value in the detection of *Strongyloides stercoralis*, since the larvæ of this species, which in this case are found already hatched in the stools, are also not floated. The eggs of *Enterobius vermicularis* are rarely found in routine fecal examinations,

but this species offers little difficulty in diagnosis on account of the anal itching and the finding of mature females.

Special Literature on Fecal Diagnosis

Hall, M. C. 1911. A Comparative Study of Methods of Examining Feces for Evidence of Parasitism. Bull. 135, Bureau of Animal Industry, U. S. Department of Agriculture.

Howard, H. H. 1919. The Control of Hookworm Disease by the Intensive Method. Publ. No. 8, International Health Board, Rockefeller Foundation, pp. 55–68.

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Kofoid, C. A. and Barber, M. A. 1918. Rapid Method of Detection of Ova of Intestinal Parasites in Human Stools. Jour. Amer. Med. Assoc., Vol. 171, pp. 1557–1561.

6. Identification of Eggs in Feces

A. *General Discussion.* Certain general considerations are necessary before specifically taking up the diagnosis of the worms of man from their eggs in the feces. The beginner will often confuse various vegetable cells, pieces of mucus, etc., found in feces, but the regular clear-cut outline of the shell of the parasite eggs usually makes the determination easy. If a worker will become thoroughly familiar with a few of the more important types of worm eggs, he will soon eliminate this difficulty. In fact, he will soon find that he can instantly recognize the eggs of the species of worms commonly found in man in the region where he is working. Now and then,

however, he will run across a type of egg or worm, usually a small nematode, which he has difficulty in identifying. In the case when an egg is found which is difficult to identify he can first examine it to decide whether it is a trematode, cestode or nematode egg, and compare it with the figures of the eggs from human feces. If he cannot readily identify it, there are certain other possibilities which must be considered. Mite eggs in human feces have sometimes been confused with nematode eggs. If the doubtful egg is of this type, it will usually be possible to find eggs far enough advanced so that the arthropod character of the enclosed embryo will be clear. It is not at all unusual for men to find worm eggs which do not agree with any which have been reported in human feces. Eggs of the parasites of lower animals or of free-living nematodes may contaminate the food of man and pass through the digestive tract uninjured. Such a possibility can usually be eliminated in the case of an unknown egg by a later examination separated by several days from the first. There is also the possibility that such an egg belongs to a parasite of some lower animal which has incidentally infested man, or even to a new human parasite. There are constantly being reported new parasites of man, and it is probable that as new regions are explored or regions already studied, further explored, this number will constantly increase. Field workers and physicians who find eggs which they cannot identify will render a service to science if they will send them to special-

ists. The finding of free-living nematodes which have contaminated fecal samples is also a source of confusion. Certain species of free-living nematodes have also been reported as temporary parasites of the human intestine.

The following key and plate of eggs (Pl. VII) are included with the hope that they will be of assistance to workers in learning to identify the eggs of the common human helminths.

B. Key for the Identification of the Eggs of Human Helminths

A. Eggs in urine.

Schistosoma hæmatobium (Pl. VII, 3). Large terminal spine; contain fully developed miracidium; 120 μ to 150 μ by 40 μ to 60 μ.

B. Eggs in sputum.

Paragonimus westermani[1] (Pl. VII, 5). Operculate; brownish or yellowish brown in color; contains fertilized ovum surrounded by yolk cells; 80 μ to 100 μ by 52 μ to 75 μ.

C. Eggs in feces.

I. Operculum present.

a. Eggs under 40 μ in length; contain a fully developed miracidium.

1. Operculum sharply defined with shell projecting slightly behind its edge.

(a) *Opisthorchis felineus*.[2] Size of egg 30 μ by 11 μ.

(b) *Clonorchis sinensis*.[2] (Pl. VII, 6). Average size of egg, 29 μ by 16 μ.

[1] Eggs of this form are also found in feces from swallowing of sputum by the patient.

[2] It is difficult in the present state of our knowledge to distinguish these species by their eggs. Since their geographical distribution differs, the locality from which the patient comes will usually give a clue to the specific identity of the worms.

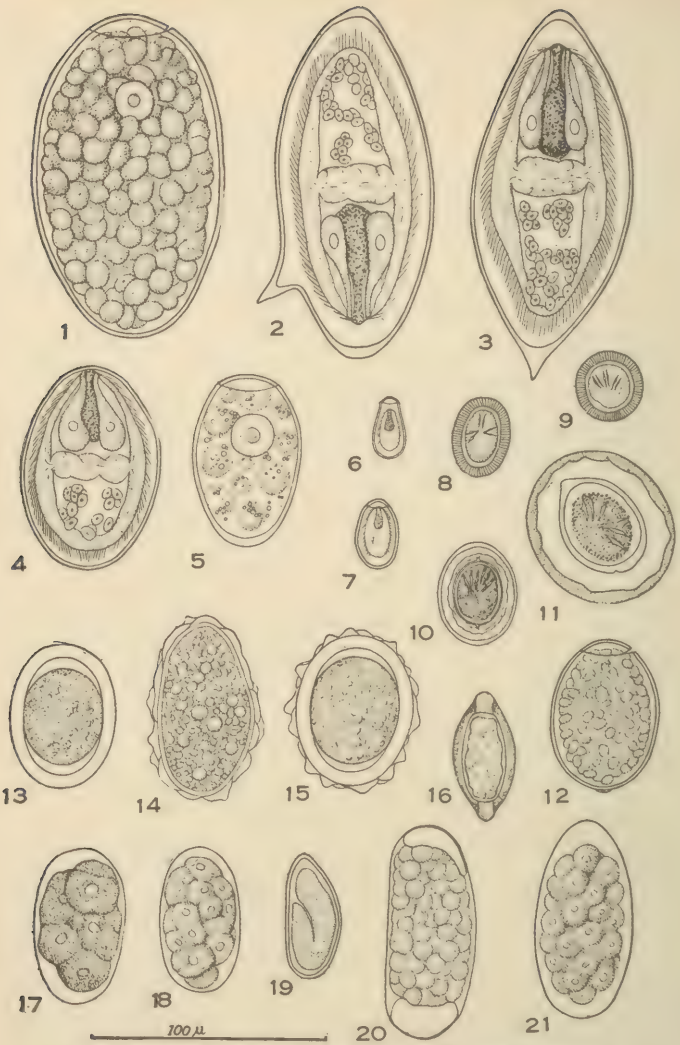

PLATE VII. — EGGS OF THE MOST IMPORTANT HUMAN HELMINTHS

(All the drawings are original except figures 7, 8, 9, and 11, which are modified from other authors. All the figures are at the same magnification.)

1. Fasciolopsis buskii. 2. Schistosoma mansoni. 3. Schistosoma hæmatobium. 4. Schistosoma japonicum. 5. Paragonimus westermani. 6. Clonorchis sinensis. 7. Metagonimus yokogawai. 8. Tænia saginata. 9. Tænia solium. 10. Hymenolepis nana. 11. Hymenolepis diminuta. 12. Diphyllobothrium latum (Dibothriocephalus latus). 13. Ascaris lumbricoides (egg without outer coating). *14. Ascaris lumbricoides* (abnormal egg). *15. Ascaris lumbricoides. 16. Trichuris trichiura. 17* and *18. Hookworm eggs. 19. Enterobius vermicularis* (*Oxyuris vermicularis*). *20. Oxyuris incognita. 21. Trichostrongylus orientalis.*

2. Operculum not sharply defined, the shape being regularly oval.
   (a) Egg brownish with thick shell. *Heterophyes heterophyes*. Average size of eggs 30 μ by 17 μ.
   (b) Egg yellowish with rather thin shell. *Metagonimus yokogawai* (Pl. VII, 7). Average size of eggs 28 μ by 16 μ.

b. Eggs over 50 μ in length; do not contain a fully developed embryo.
   1. *Paragonimus westermani*[1] (Pl. VII, 5). Size of egg 80 μ to 100 μ by 52 μ to 75 μ.
   2. *Fasciolopsis buskii* (Pl. VII, 1). Size of egg 120 μ to 130 μ by 77 μ to 80 μ.
   3. *Gastrodiscoides hominis* (*Gastrodiscus hominus*). Size of egg 150 μ by 72 μ.
   4. *Diphyllobothrium latum* (*Dibothriocephalus latus*) (Pl. VII, 12). Size of egg 55 μ to 76 μ by 41 μ to 56 μ; operculum small, not sharply defined. Shell thin, transparent, light straw color.

II. Operculum absent.

a. Round or slightly oval, containing a six-hooked embryo; cestode eggs.
   1. With a thick radially striated, inner shell or embryophore.
      (a) *Tænia saginata*[2] (Pl. VII, 8). Embryophore, ovoid, rusty brown, 35 μ to 40 μ in length by 20 μ to 30 μ in width.
      (b) *Tænia solium* (Pl. VII, 9). Embryophore almost round; brown, 31 μ to 36 μ in diameter.

[1] The eggs of this species are found only occasionally in feces, since in man they are usually expelled with the sputum.

[2] It is extremely difficult to distinguish between the eggs of T. solium and T. saginata in feces, but whole proglottids will usually be found, which are easily distinguished (Pl. X, 3a and d).

2. With thin membranous inner shell.
   (a) *Hymenolepis nana* (Pl. VII, 10). Oval or globular with two distinct membranes; outer 30 μ to 60 μ in diameter; inner 16 μ to 34 μ, filiform projections at each pole of inner membrane.
   (b) *Hymenolepis diminuta* (Pl. VII, 11). Round or oval; outer membrane 54 μ to 86 μ; yellowish, may be striated; inner membrane 24 μ to 40 μ by 36 μ.

b. Oval, yellowish brown in color containing a fully developed miracidium; trematode eggs.
   1. Large lateral spine present, 120 μ to 140 μ by 50 μ to 60 μ *Schistosoma mansoni.* (Pl. VII, 2).
   2. Only small rudimentary spine, if any present, 60 μ to 85 μ by 35 μ to 40 μ. *Schistosoma japonicum.* (Pl. VII, 4).

c. Shape, oval, considerably longer than wide; nematode eggs.
   1. Somewhat barrel shaped; with plugs at each end. *Trichuris trichiura* (Pl. VII, 16). Color of eggs dark brown; 50 μ to 57 μ by 23 μ; ovum unsegmented.
   2. Thick transparent shell with an external albuminous coating which forms protuberances. *Ascaris lumbricoides* (Pl. VII, 13, 14, 15). Color of eggs brown; 50 μ to 70 μ by 40 μ to 50 μ; ovum unsegmented.
   3. Asymmetrical, flattened on one side.
      (a) *Enterobius vermicularis* (Pl. VII, 19). Color transparent; contains tadpole like embryo; size of egg 50 μ to 55 μ by 16 μ to 24 μ.

(b) *Oxyuris incognita* (Pl. VII, 20). Oil globules at each end of egg; average size of egg 95 μ by 40 μ.

4. Shell thin, transparent; embryo in four to many cell stages.
   (a) *Trichostrongylus orientalis* (Pl. VII, 21). Ends somewhat pointed; size 75 μ to 90 μ by 39 μ to 47 μ.
   (b) *Necator americanus*. Size 58 μ to 80 μ by 35 μ to 52 μ.
   (c) *Ancylostoma duodenale* (Pl. VII, 17–18). Size 56 μ to 61 μ[1] by 34 μ to 38 μ.

7. Identification of the Free-living Stages of *Strongyloides Stercoralis* and the Hookworm

In certain types of experimental work it becomes very important to be able to identify the larvæ and free generations of *Strongyloides stercoralis* and the different larval stages of the hookworm. Since the eggs of *Strongyloides stercoralis* hatch in the human intestine, the diagnosis of this species rests upon the recognition of the first larval stage. This form in a fresh fecal sample (Pl. VIII, 8) varies in length from 200 to 250 microns and in width from 13 to 15 microns. The buccal cavity is short, having a length of less than one half the diameter of the body. The genital anlage is large and conspicuous, situated at the side of the intestine just back of the middle of the body and has a length of about 30 microns.

[1] Since the size ranges of the two species of hookworm eggs overlap it is extremely difficult to distinguish them, especially as in many regions both species are present.

If feces is kept for more than twenty-four hours before examination, hookworm eggs will have had time to hatch and it will be necessary to distinguish the newly hatched hookworm larva from strongyloides larvæ. Two points of difference are most helpful in making this discrimination (cf. Pl. VIII, 8 and 10). The hookworm larva has a much longer and more distinct buccal cavity than the strongyloids larva. It also has a very small inconspicuous genital anlage of 4 to 5 microns in length.

The infective or filariform larva of *Strongyloides stercoralis* (Pl. VIII, 9) is long and slender and is easily distinguished by the fact that its long slender esophagus has a length equal to almost half the length of the body. The tip of the tail has a distinct cleft, which, however, can only be seen with the high power of the microscope.

In certain types of experimental studies on hookworm disease it becomes a matter of great importance to be able to distinguish the infective hookworm larvæ from the infective larvæ of the parasites of animals, and from free-living nematodes. Figure 11 of Plate VIII is the infective larva of *Ancylostoma duodenale* and Plate IX gives a series of microphotographs of the infective larvæ of *Necator americanus*. The best way to learn to readily identify these hookworm larvæ is to study them carefully from known cultures. It is extremely difficult and not necessary for most work to distinguish the larvæ of *Ancylostoma duodenale* from those for *Necator americanus*. In identifying hookworm

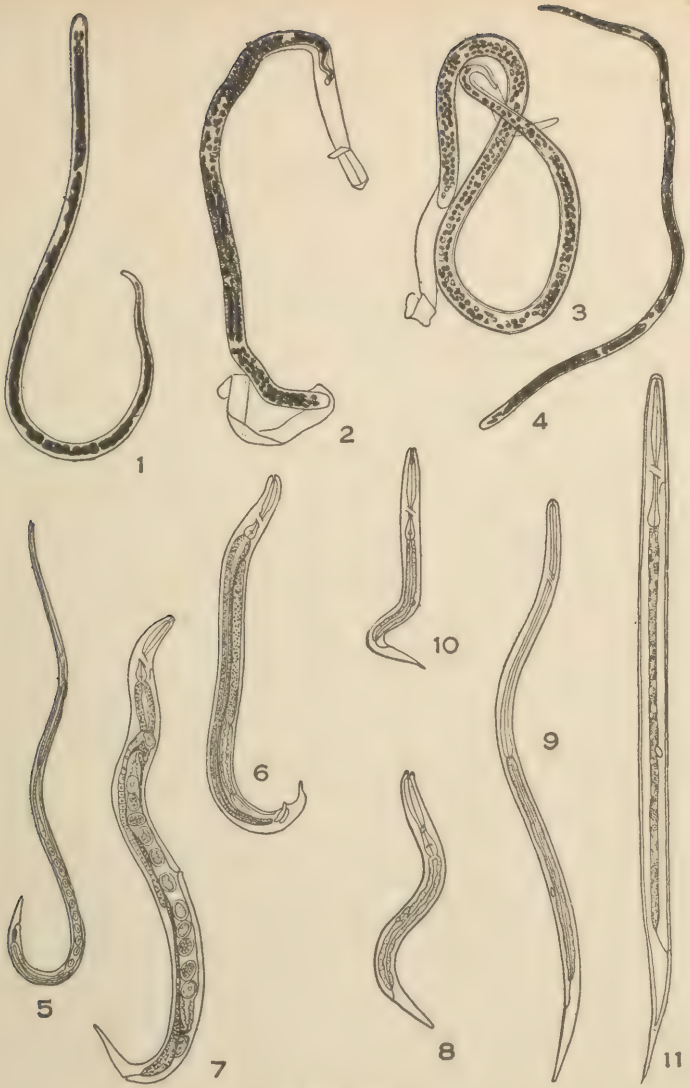

PLATE VIII

1. Larval stage of *Filaria ozzardi* (*F. demarquayi*). After Fulleborn. *2*. Larval stage of *Loa loa* (*Microfilaria diurna*). After Fulleborn. *3*. Larval stage of *Filaria bancrofti* (*Microfilaria nocturna*). After Fulleborn. *4*. Larval stage of *Acanthocheilonema perstans* (*Microfilaria perstans*). After Fulleborn. (Figs. *1–4*, × 450.) *5*. Adult parasitic female of *Strongyloides stercoralis*. After Looss. (× 35.) *6* and *7*. Adults, male and female, of the free living generation of *Strongyloides stercoralis*. After Looss. (× 75.) *8*. Rhabditiform larva of *Strongyloides stercoralis* just hatched from the egg. After Looss. (× 125.) *9*. Filariform infective larva of *Strongyloides stercoralis*. After Looss. (× 125.) *10*. Rhabditiform larva of *Ancylostoma duodenale* just hatched from the egg. After Looss. (× 125.) *11*. Filariform infective larva of *Ancylostoma duodenale*. After Looss. (× 125.)

larvæ it must be kept in mind that in the filariform infective stage they may vary from about 0.4 to 0.7 mm. in length, may be either sheathed or unsheathed, and may offer considerable variation in

PLATE IX. — INFECTIVE LARVÆ OF NECATOR AMERICANUS.

1. Sheathed larva containing many food granules. *2*. Sheathed larva with considerable reduction of food granules. *3*. Sheathed larva with food granules much reduced. *4*. Unsheathed larva with food granules much reduced.

appearance due to the physiological age, i.e., the amount of stored food material present, which produces differences in appearance. Plate IX was prepared to show these variations for the infective larvæ of *Necator americanus*.

8. The Culture of Feces as a Method of Diagnosis and for Obtaining Larval Stages

A. *Looss Bone-black Method of Culturing Hookworm larvæ.* The Looss method for culturing hookworm larvæ has been utilized in several hookworm campaigns for the purpose of diagnosis. The method used was to mix the fecal sample with bone black and incubate in a petri dish at about 25–30 C. for five or six days. It was necessary to keep the culture well moistened during this time. The cultures were then flooded with water, which was removed after two to three hours and examined for infective hookworm larvæ. This is probably the most accurate of all routine methods which have been used for the diagnosis of hookworm infestation. Under most circumstances it is not a practical method on account of the amount of manipulation necessary and the length of time which must elapse from the securing of the sample until the diagnosis can be made. This is, however, the standard method for obtaining infective hookworm larvæ for study or experimentation. It has been found that under tropical conditions, especially when large numbers of larvæ are needed for experimental work, this material is not always satisfactory, on account of the ease with which the cultures become contaminated with free-living nematodes.

B. *Ackert's Ashes-soil Method of Culturing.* Ackert devised a more satisfactory method of culturing hookworm larvæ while carrying on investigations in

Trinidad. This method can only be used in connection with the apparatus for isolating infective hookworm larvæ from the soil which will be described in a later section. As a culture medium soil was used which had been heated to a temperature of 150° F. It was placed to a depth of about 2 inches in shallow tin pans in which holes had been punched to secure drainage. To make the culture the stool should be spread out in a layer about one-fourth of an inch in thickness on the soil and covered to a depth of about 1 mm. with wood or charcoal ashes. The sprinkling of the ashes over the stool inhibits the growth of mold and also prevents the formation of a hard crust. The cultures should be kept in a screened place, protected from rats and other animals and kept well moistened. After a period of 5 to 8 days the top half inch of the culture should be scraped off and placed in the isolation apparatus. By this method large numbers of hookworm larvæ can usually be obtained.

C. *A Soil-culturing Method.* The use of the ashes, however, is not necessary for obtaining good cultures. Very large numbers of larvæ can usually be obtained by simply mixing feces containing hookworm eggs with sterilized sand or humus. The feces should be thoroughly mixed with about three times their weight of soil and spread out in a shallow pan to the depth of about 1 inch. The culture should be kept constantly moistened. After about 5 to 9 days, according to the temperature, larvæ can be isolated from these cultures. At shorter intervals

after culturing the early stages of development of the hookworm larvæ can be isolated. It is possible by planning the time intervals to obtain large numbers of all the stages of development of the hookworm larvæ from such cultures. Strongyloides larvæ can also be cultured by either of the above methods.

D. *Culture Method for the Diagnosis of Strongyloides stercoralis.* Darling has recommended the use of the culture method for the diagnosis of *Strongyloides stercoralis.* The larvæ of this parasite can be directly distinguished from newly hatched hookworm larvæ as indicated above or the larvæ of free-living nematodes which have been introduced by contamination, by careful microscopical comparison. But in doubtful cases a certain diagnosis can be made by culturing to obtain the infective larvæ, which are very characteristic and easily recognized, or the free-living adult stages (Pl. VIII, 6, 7 and 9).

9. Special Methods for Investigational and Control Work on Hookworm Disease

A. *Stoll's Method for Counting the Number of Eggs in Feces.* Stoll, in connection with investigations on hookworm disease, has devised a simple method of counting helminth eggs in stools. The steps in this technique are as follows:

1. Weigh by difference 3 grams of the feces to be examined into a large-sized test tube or centrifuge tube graduated at 45 cc.

2. Pour in a decinormal solution of sodium hydroxide up to the 45 cc. mark.

3. Add ten small (3 mm.) glass beads, close the tube with a rubber stopper and shake until a homogeneous suspension has been obtained.

4. Immediately transfer 0.15 cc. of the material with a pipette, graduated to 0.15 cc., to a 2 x 3 inch slide and cover it with a 22 x 40 mm. No. 2 cover slip.

5. Count the total number of eggs in this preparation, using a mechanical stage, and multiply by 100 to give the number of eggs per gram of feces.

Certain points in this technique will be explained somewhat further. Weighing by difference is speedy and accurate and entails but little exposure of the feces to air. Two 3-gram samples can be weighed out from each specimen after a single balancing of the scales, and the average of the two counts used. The use of decinormal sodium hydroxid tends to saponify the fats in the feces, and apparently makes the hookworm eggs less sticky. This solution gives a much more uniform suspension than any other tried. Samples of 0.15 cc. of the mixture indicated give a high degree of visibility, and so expedite the microscopical examination. Stoll, after using this method in a large number of counts, and subjecting it to a variety of tests came to the conclusion that in routine practice it yields average counts correct to within less than 10 per cent of the absolute number of eggs present in the fecal specimens.

This method is of value in a variety of experimental work. For example, it makes it possible to determine how many hookworm eggs are present in

any specimen of feces cultured to obtain hookworm larvæ, and places experimental work on the factors influencing the development of hookworm larvæ on a quantitative basis. It also gives a sufficiently close approximation of the amount of infestation of an individual so that it has been used effectively in field studies or hookworm control campaigns to determine the degree of infestation of a population.

B. *Darling's Worm-count Method.* Darling and his co-workers have devised a worm-count method which they have used extensively in hookworm investigations in determining the relative value of different anthelminthics and in discovering the degree of infestation and the worm index. This method has a wide value in various types of studies connected with hookworm control and so will be described here in some detail.

A hookworm patient from whom a count is to be made is given a standard treatment and all feces subsequently passed are kept for 48 hours or more. Great care must be exercised to obtain all the feces passed, and not to get the specimens mixed if several people are being counted at one time. A regular routine is followed in the washing of stools. Those that are soft can be washed at once while those which are more compact must be mixed with water and stirred until soft. The washing is done by means of a jet of water played into a large brass wire sieve (with a mesh of 50 to an inch), into which the contents of the vessel in which the feces have been mixed is poured. It is advisable to use a stream

of moderate force in order not to wash the worms through the sieve. The washed stool is next distributed into photographic development trays. A dark brown tray is found to give the best background for locating the worms. The worms are then picked out with a needle or forceps and placed in properly numbered petri dishes containing normal salt solution. At least four stools should be collected from each person. But the number to be examined from a given patient will usually be greater than that, for no case is considered complete until two consecutive negatives have been obtained. Finally, the excess salt solution is drained from the petri dishes, the worms killed in hot 70 per cent alcohol, differentiated into species and sexes and counted. Before a count is considered satisfactory for any given patient, he is treated again one week after the first treatment and the worms expelled counted in the same manner.

C. *Baermann's Apparatus for Isolating Infective Hookworm Larvæ from Soil.* A simple apparatus invented by Baermann makes it possible to isolate nematodes from considerable quantities of soil. This apparatus is of value in investigations of soil nematodes and those of the parasitic species which have developmental stages in the soil. It is of value in studying soil infestation with hookworm larvæ, in determining the sources of human infestation and in various types of investigations on the activities of the infective larvæ. A description of this apparatus and its method of use follows.

The Baermann apparatus for the isolation of hookworm larvæ from the soil works by bringing the lower surface of a soil sample into contact with water of a considerably higher temperature. Under these conditions a large proportion of the nematodes in the sample will pass into the water and can be collected and counted. This isolation apparatus consists of a glass funnel almost filled with water, which has the outlet closed by a clamped piece of rubber tubing. The soil sample is placed in a sieve, which is then fitted down into the funnel so that the level of the water is above the surface of the soil. For the examination of soil samples of half a pint or more large glass funnels 9 inches in diameter and specially prepared brass sieves 7 inches in diameter and 3 inches in height, with a 1 mm. mesh, should be used. To prevent small particles of soil from sifting through into the funnels the sieves are lined with one or two thicknesses of cloth. Tightly fitting rubber tubes closed with Hoffman clamps are placed on the stems of the funnels. For convenience, supports of suitable height can be made, and the funnels held in place by padded cross bars. It is possible to substitute for the sieve a piece of wire screen covered with cloth, of such a size that it can be fitted down into the funnel. The sieves have the advantage of being more easily handled in the transfer of soil.

In routine procedure the soil should be thoroughly broken up before being emptied into the sieves. Precautions should be taken not to carry over a

little of the soil from one sample to another, and to keep the bottom of the sieve from contact with any dirt. The water used in the funnels should be heated to a temperature of about 115° F. A funnel, after being set up, is filled within an inch and a half of the rim, so that when the sieve is placed in position there will be no contact between the water and the bottom of the sieve. Water of the same temperature is then poured over the rim of the funnel, until it is practically full and the level of the water above the level of the soil. The samples can be set up in the isolation apparatus some time during the day and left standing over night before the examinations are made. The method of drawing off the water is to open the clamp at the bottom of the rubber tube and let the water run out into a 50 cc. centrifuge tube. After centrifugation the supernatant water is drawn off with a long pipette and the residue spread out on a 2 x 3 inch slide, for microscopical examination. In some instances, when the soil sample is of saturated clay or very finely divided sand, a considerable amount of sediment may fall into the funnel. This can, if necessary, be reduced in amount by running it through a smaller isolating apparatus before microscopical examination.

For the examination of small soil samples it is convenient to use a funnel about 9 cm. in diameter. Pieces of screen, bent into a cone shape and lined with cloth, can be used for holding the soil samples. The smaller apparatus effects a considerable saving of time in the examination of small samples.

Literature on Special Methods

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Darling, S. T., Barber, M. A. and Hacker, H. P.: 1920. Hookworm and Malaria Research in Malaya, Java, and the Fiji Islands. Pub. No. 9, International Health Board, Rockefeller Foundation, New York, pp. 23–31.

Stoll, N. R.: 1923. Investigations on the Control of Hookworm Disease, XV: An effective method of counting hookworm eggs in feces. Amer. Jour. Hygiene, Vol. III, pp. 59–70.

10. Identification of Adult Worms Found in Feces

No attempt will be made in this bulletin to describe the structure or life history of the worms parasitic in man. For this information the student is referred to the textbooks in Parasitology and Tropical Medicine listed at the first of this bulletin. There are, however, certain discriminations that the physician or field worker may be called on to make. The presence of *Tænia solium* and *Tænia saginata* is usually diagnosed by the finding of the ripe proglottids in the stools. It is of interest and importance that the physician should know which of these forms he is dealing with. The following comparison with the figures (Pl. X, 3b and 3d) will make it easy to decide this point.

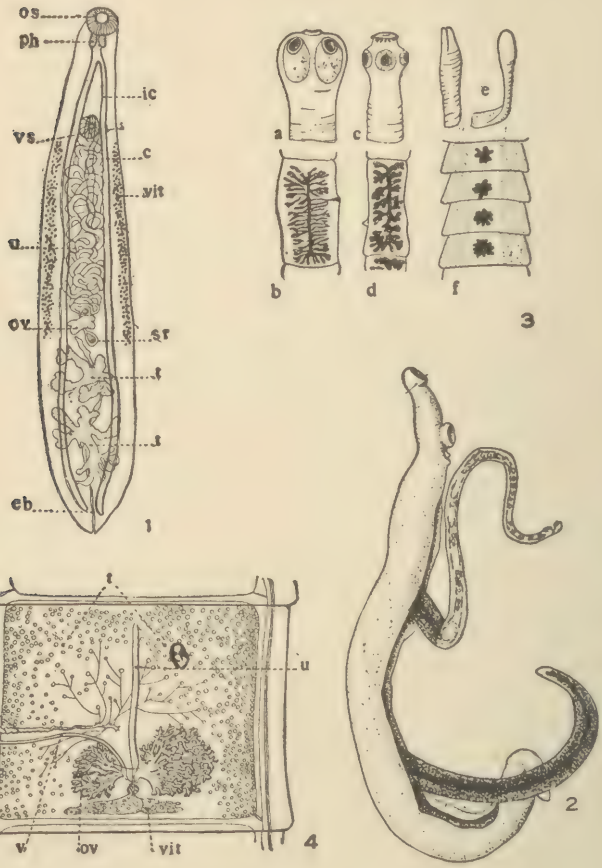


PLATE X.

1. Adult of *clonorchis sinensis*. From Kobayashi. Letters used: *c*, cirrus sac; *eb*, excretory bladder; *ic*, intestinal cecum; *os*, oral sucker; *ov*, ovary; *ph*, pharynx; *sr*, seminal receptacle; *t*, testis; *u*, uterus; *vs*, ventral sucker; *vit*, vitellaria. *2*. Adult male and female of *Schistosoma japonicum* in copula. After Looss. *3*. Scolices and ripe proglottids of cestodes; *a*, scolex of *Tænia saginata*; *b*, ripe proglottid of *T. saginata*; *c*, scolex of *T. solium*; *d*, ripe proglottid of *T. solium*; *e*, scolex of *Diphyllobothrium latum* (*Dibithriocephalus latus*); *f*, proglottids of *D. latum*. From Hertwig after Leuckart, Braun, and Schauinsland. *4*. Mature proglottid of *Tænia saginata*. After Sommer. Letters used: *c*, cirrus sac; *ov*, ovary; *t*, testes; *u*, uterus; *v*, vagina; *vit*, vitellarium.

Tænia solium (Pl. X, 3c and 3d)	Tænia saginata (Pl. X, 3a and 3b)
Scolex globular about 1 mm. in length	Scolex quadrangular 1.5 to 2 mm.
Rostellum with two crowns of hooks	Rostellum and hooks absent
Length 2–8 meters	Length 4–12 meters
Number of proglottids 700–1000	Number of proglottids about 2000
Genital pores irregularly alternating	Genital pores more regularly alternating
Branches of uterus in gravid proglottis 5 to 10 in number and dendritic	Branches of uterus in gravid proglottis 15–30, dichotomous
Proglottids expelled in groups passively with feces	Proglottids expelled singly and may force anal sphincter
Larval form *Cysticercus cellulosæ* of the pig, sometimes in man	Larval form *Cysticercus bovis* in cattle, never found in man

In connection with hookworm treatment and investigations it is often important to be able readily to distinguish between *Ancylostoma duodenale* and *Necator americanus*. A trained observer can learn to distinguish between these two species with the naked eye or a low-power lens. The most significant characteristic for such a determination is the sharp bend at the anterior end of Necator americanus and the fact that this species has a buccal capsule which is smaller in proportion to the size of the body than *Ancylostoma duodenale*. Figures 1 to 4 in Plate XI will make possible an accurate discrimination between these species on microscopical examination. Figures of several other adult worms are included in Plates X and XI, to give the anatomy of groups of parasitic worms.

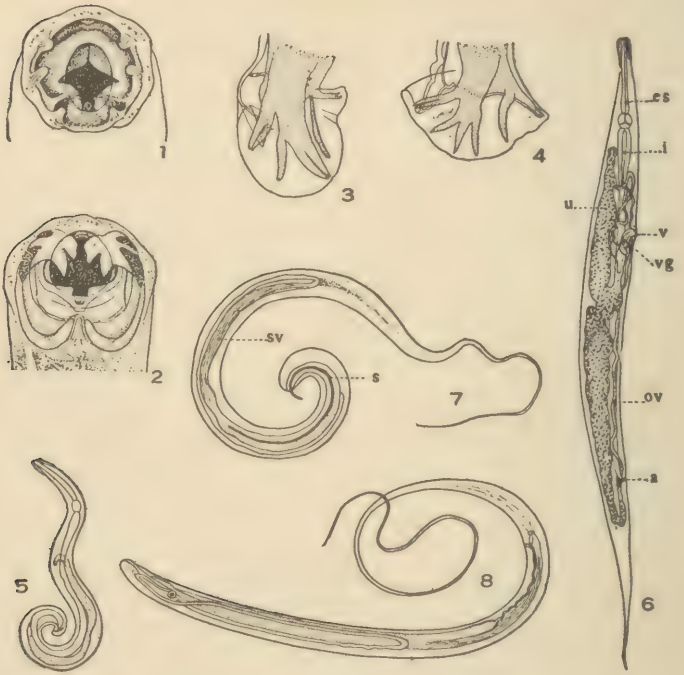


PLATE XI.

1. Anterior end of *Necator americanus*. From Looss. *2*. Anterior end of *Ancylostoma duodenale*. From Looss. *3*. Bursa of *Necator americanus*. From Looss. *4*. Bursa of *Ancylostoma duodenale*. From Looss. *5*. Adult male of *Enterobius vermicularis* (*Oxyuris vermicularis*) From Leuckart. *6*. Adult female of *Enterobius vermicularis* (*Oxyuris vermicularis*). From Leuckart. Letters used: *a*, anus; *es*, esophagus; *i*, intestine; *ov*, ovary; *u*, uterus; *v*, vulva; *vg*, vagina. *7*. Adult male of *Trichuris trichiura*. From Castellani and Chalmers after Claus. Letters used: *s*, spicule; *sv*, seminal vesicle; *t*, testis. *8*. Adult female of *Trichuris trichiura*. From Castellani and Chalmers after Claus.

11. Methods of Preserving and Mounting Parasitic Worms

The physician or field worker will often want to preserve or make permanent mounts of parasitic worms either for his own study or to send to special-

ists. I am including, therefore, a brief description of certain of the methods of technique which will be helpful in this connection.

Nematodes may be killed by dropping into a hot (not boiling) solution of 70 per cent alcohol plus 10 per cent glycerin. They can be left in this same solution for preservation.

Trematodes and Cestodes. Small trematodes can best be killed by Looss' shaking method. Shake vigorously for three minutes in small amount of salt solution (.4 to .5 per cent). The killing solution (saturated aqueous solution of corrosive sublimate plus 2 per cent acetic acid) is then poured on cold. Keep in the killing solution from 4 to 8 hours. Then wash in 30 per cent alcohol one half hour and 50 per cent alcohol one half hour and transfer to 70 per cent alcohol which is tinged with iodin. Change to 70 per cent alcohol and wash until color of iodin is removed and preserve in 80 percent alcohol plus 5 per cent glycerin.

Large trematodes and cestodes may be killed by pouring hot killing solution over them when they are flattened between slides. Small to medium-sized cestodes can be stretched out by allowing them to hang from a toothpick. The killing solution is then poured down along their length.

Five per cent formalin can be used for a killing and preserving medium for trematodes and cestodes when it is impossible to carry through the long technique.

Parasite eggs in feces can be preserved satisfactorily in 5 per cent formalin.

Pathological tissues showing the effects of para-

sitic worms can be fixed in 10 per cent formalin and preserved in 70 per cent alcohol.

It is usually sufficient for the examination of nematodes to allow the alcohol to evaporate and to study them in the glycerin medium mounted temporarily on a slide and covered with a cover glass. In such preparations the worm is usually sufficiently clear for study and can be rolled around for the examination of points necessary for identification.

For trematodes and cestodes the following technique is recommended for the preparation of the worms for examination.

Technique for making toto mounts of Trematodes and Cestodes

1. Specimens preserved in 80 per cent alcohol plus 5 per cent glycerin.
2. 70 per cent alcohol 30 min. to 1 hour.
3. 50 per cent alcohol 30 min. to 1 hour.
4. 30 per cent alcohol 30 min. to 1 hour.
5. Distilled water.
6. Stain in very dilute Delafield's Haematoxylin 4–24 hours.
7. Wash in distilled water.
8. 30 per cent alcohol 30 min. to 1 hour.
9. 50 per cent alcohol 30 min. to 1 hour.
10. 70 per cent alcohol 30 min. to 1 hour.
11. Destain in 80 per cent alcohol plus 1 to 2 per cent HCl until properly differentiated.
12. Blue in 80 per cent alcohol with an alkaline reaction.
13. Flatten between two slides separated with small strips of cardboard and tied with thread. (Pass through the higher grades of alcohol while still between the slides.)
14. 95 per cent alcohol 1 hour.
15. 100 per cent alcohol 1 to 2 hours.
16. Xylol 1 to 2 hours until specimen is entirely clear.
17. Mount in balsam, using one of the slides between which the specimen was flattened.

PART III

ARTHROPODS OF MEDICAL IMPORTANCE

PART III

ARTHROPODS OF MEDICAL IMPORTANCE

BY

FRANCIS METCALF ROOT

1. INTRODUCTION TO THE ARTHROPODS

ARTHROPODS may be defined as segmented, multicellular animals with jointed appendages and a firm, cuticular exoskeleton. The Phylum *Arthropoda* includes the organisms familiar to everyone under the common names of crabs, centipedes, insects, spiders, ticks, etc. In contrast to the *Protozoa* and the *Helminths*, Arthropods are of interest to medical men primarily on account of their röle as vectors or carriers of human diseases, although a number of Arthropods do cause human diseases by direct parasitism. The four Classes of the *Arthropoda* which contain members of medical importance may be distinguished as follows:

CRUSTACEA. Mostly aquatic, breathing by gills. Body divided into two main regions, cephalo-thorax (head plus thorax) and abdomen. Two pairs of antennæ present. Usually four or more pairs of legs.

Examples — crab, lobster, shrimp, water-flea.

MYRIAPODA. Terrestrial, breathing by tracheæ (air-tubes). Segments behind head all alike, with no differentiation into

thorax and abdomen. One pair of antennæ. Many pairs of legs.

Examples — centipede, millipede.

INSECTA. Mainly terrestrial and aerial, breathing by tracheæ. Body very definitely divided into head, thorax and abdomen in adult. One pair of antennæ. Adult with three pairs of legs and usually two pairs of wings.

Examples — bee, fly, flea, louse.

ARACHNIDA. Mainly terrestrial. Body usually divided into cephalothorax and abdomen, but in ticks and mites this division is absent. Antennæ absent. Four pairs of legs in all stages except newly hatched larvæ, where there are three.

Examples — spider, tick, scorpion.

The great majority of Arthropods of medical interest belong in the Class *Insecta*, which will therefore be considered first.

2. Class Insecta

The Class *Insecta*, including all the true insects, is divided into from nineteen to thirty-seven or more orders, according to the degree of specialization deemed desirable by the particular systematist who is drawing up the classification. The important insect disease-carriers all fall in four of these orders, which may be distinguished from each other and from all other insects by the combinations of characters listed below:

1. With functional wings in the adult stage.

   Diptera. Fore wings membranous and functional, hind wings represented by a small pair of knobbed threads of no use for flight.

   Hemiptera. Fore wings leathery at base, membranous

at tip, folded flat on top of the abdomen when at rest. Hind wings membranous throughout, folded up under the fore wings when at rest.

2. Without any vestiges of wings in the adult stage.

Siphonaptera. Body compressed laterally, hind legs modified for jumping.

Siphunculata. Body flattened dorso-ventrally, all legs modified for grasping hairs. Mouth-parts form a piercing proboscis, retracted within the head when not in use.

NOTE. — A few aberrant wingless forms of *Diptera* (e.g., *Melophagus*) and *Hemiptera* (e.g., *Cimex*) will be confusing here. They can usually be distinguished from the *Siphunculata* by their larger size.

A. *Order Diptera — the Two-winged Flies*

The *Diptera*, or true flies, include more species of medical importance than any other group of the Arthropods. The great majority of the species can at once be distinguished from all other insects by their single pair of functional wings. A few species are wingless, but fortunately none of them are of great importance to the medical man.

From the medical point of view we can distinguish by their habits three groups of flies which are of interest to us:

Blood-sucking Flies — some of which suck up disease-producing organisms from the blood of sick men and later inject them into the blood of healthy men.

Filth Flies — which have habits enabling them to pick up disease-producing organisms from human feces and deposit them again on human food.

Myiasis-producing Flies — whose larvæ may produce disturbances by living as parasites in the human body.

1. Blood-sucking Flies.

Members of many different families of the *Diptera*

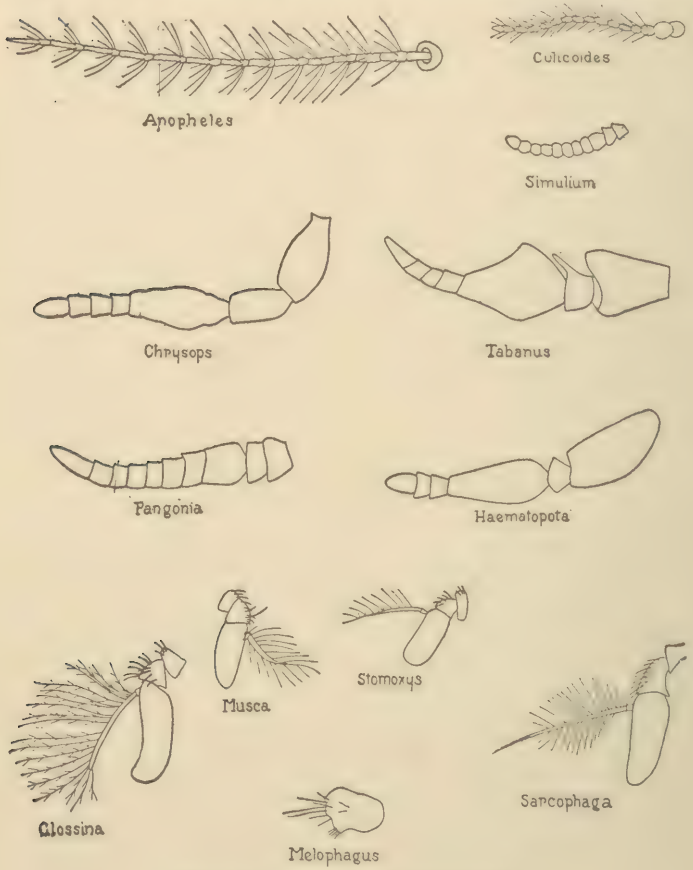


PLATE XII. — ANTENNÆ OF VARIOUS FLIES.

have developed the habit of feeding on the blood of man or the higher animals. The characters which

are particularly important for distinguishing between the different groups are the structure of the antennæ and the arrangement of the veins of the wings. Some of these differences are illustrated by the figures on Plates XII and XIII. The different groups may be tabulated as follows:

A. Antennæ long and slender, with whorls of hairs on each of the twelve or more joints (Pl. XII, *Anopheles* and *Culicoides*).

1. Family *Psychodidæ* — Moth-like Flies — Wings and body covered with long hairs, wings with numerous parallel longitudinal veins and almost no cross-veins (Pl. XIII, *Phlebotomus*).
2. Family *Chironomidæ* — Midges — Wings and body with short hairs only, longitudinal veins fewer in number (Pl. XIII, *Culicoides*).
3. Family *Culicidæ* — Mosquitoes — Wings with veins and hind margin fringed with flattened scales (Pl. XIII, *Anopheles*). Similar scales present to a greater or less extent on the body.

B. Antennæ short and stout, without whorls of hairs on the six to ten apparent joints (Pl. XII, *Simulium, Chrysops, Tabanus, Pangonia, Hæmatopota*).

4. Family *Simuliidæ* — Black-flies — Very small, thick-set flies with the front margin of the wing very strongly veined, the hind margin very weakly veined (Pl. XIII, *Simulium*).
5. Family *Tabanidæ* — Horse-flies — Large flies with more complicated wing venation (Pl. XIII, *Chrysops*). The antennæ really have only three joints, but the third joint is long and divided into a number of segments. The squamæ (little wing-like expansions on the side of the thorax, just below the base of the wing) are rather large and conspicuous.

C. Antennæ of three joints, the third joint longer than the first

two and bearing an arista or feathered bristle on its dorsal surface near the base (Pl. XII, *Glossina*, *Musca*, *Stomoxys*, *Sarcophaga*).

6. Family *Muscidæ* — House Flies — Wing venation reduced, as in most of the higher flies (Pl. XIII, *Glossina*, *Stomoxys*). Squamæ comparatively large. Antennal arista feathered to tip on one or both sides. First posterior cell (area at tip of wing between third and fourth longitudinal veins) slightly or decidedly narrowed at the wing margin.

D. Antennæ of a single joint only, concealed in pits on the dorsal surface of the head (Pl. XII, *Melophagus*).

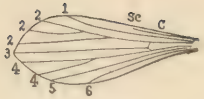
7. Family *Hippoboscidæ* — Louse Flies — Wing veins concentrated along front margin. Adult flies of this family live as ectoparasites on birds and mammals. Some genera are wingless.

1. Family *Psychodidæ* — Genus *Phlebotomus*.

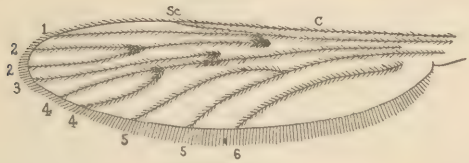
Phlebotomus, the single blood-sucking genus of this family, can always be identified by the characteristic wing venation (Pl. XIII). In all the *Psychodidæ* the second longitudinal vein has three branches (only two in most flies). In the typical *Psychodidæ*, which are not blood-sucking forms, the first branching of this vein occurs close to the base of the wing, while in *Phlebotomus* the first branching takes place in the middle of the wing. Many species of the genus have been described in recent years. The male genitalia, which are very large and include three pairs of claspers, furnish the best characters for the identification of species. A good synopsis of a number of the species is found in the paper by Miss Summers in the *Journal of the London School of*

Culicoides

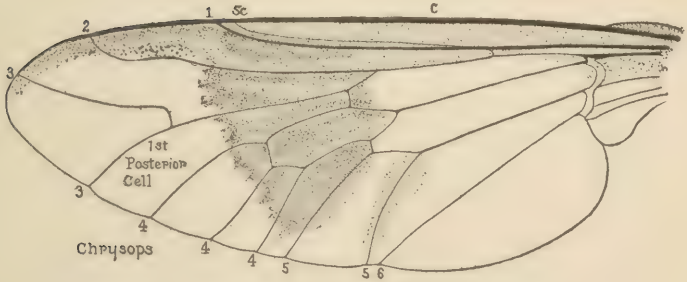
Simulium



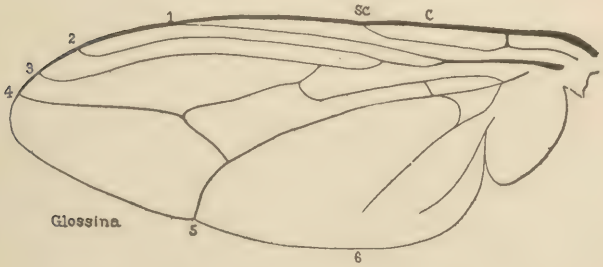
Phlebotomus



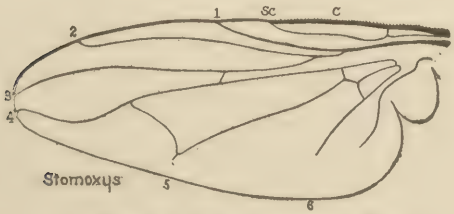
Anopheles



Chrysops



Glossina



Stomoxys

PLATE XIII. — WINGS OF VARIOUS FLIES.

Tropical Medicine, Vol. 2, pt. 2. See also numerous papers by Newstead in the *Bulletin of Entomological Research* and elsewhere.

The early stages are very minute and difficult to discover. They occur on the walls of cisterns and privy pits, in the interstices of stone walls and rock piles, in caves and in cracks in the soil.

Phlebotomus pappatasii is known to be the vector of three-day fever (Pappataci fever, sand-fly fever) in the Mediterranean region. Townsend believes that *P. verrucarum* is the vector of Verruga Peruviana in Peru. Various other species of *Phlebotomus* have been suspected as carriers of cutaneous leishmaniasis in the Orient and in South America.

2. Family *Chironomidæ* — Subfamily *Ceratopogoninæ*.

The subfamily is characterized as follows: Thorax not projecting over head. Sternopleuræ not enlarged. Antennæ with 13 to 15 joints in both sexes. Second longitudinal vein absent. Fourth longitudinal vein two-branched. Proboscis chitinized.

The following genera probably include practically all of the blood-sucking species:

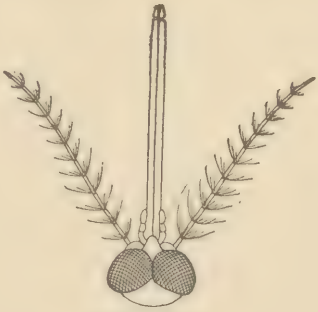
Culicoides — Antennæ with 15 joints; wings sparsely hairy, usually dappled; thorax with an anterodorsal pair of slit-like or circular depressions; anterior cross-vein distinct.

Dasyhelea — Much like *Culicoides* but without the anterodorsal depressions on the thorax.

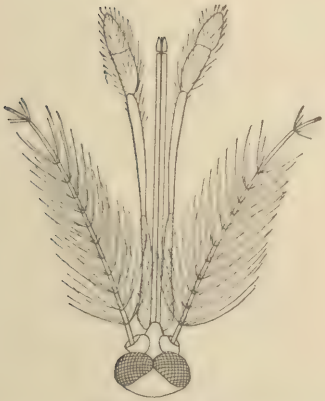
Lasiohelea — Wings densely hairy; cell at tip of wing between first and third longitudinal veins very long and narrow.

Leptoconops — Antennæ with 13 or 14 joints; costa, subcosta and first longitudinal vein very short and strongly

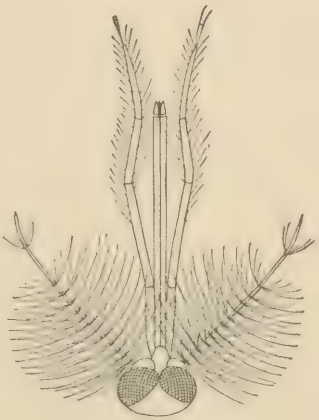
Female Anopheles



Female Culex



Male Anopheles



Male Culex

PLATE XIV. — HEADS OF MALE AND FEMALE CULICINE AND ANOPHELINE MOSQUITOES.

chitinized, other wing veins very delicate; anterior cross-vein absent; frons (upper part of face between eyes) bare or with only one pair of bristles.

Acanthoconops — Antennæ with 14 joints; much like *Leptoconops* but frons covered with bristles.

These blood-sucking midges or "sand-flies" are very annoying, but are not known to carry any disease. Their minute worm-like larvæ occur in water, mud and decaying vegetable matter.

Some of the non-blood-sucking *Chironomidæ* resemble mosquitoes as pupæ and adults and may be confused with them. They lack the characteristic wing scales and biting proboscis of the mosquitoes.

3. Family *Culicidæ* — Subfamily *Culicinæ*.

True mosquitoes can always be recognized by the scaled wing veins and the long proboscis. Most authors include a few non-biting forms in this family, making the true mosquitoes constitute the subfamily *Culicinæ*. This subfamily is divided into two Tribes, the *Anophelini* (malaria-carrying mosquitoes) and the *Culicini* (non-malaria-carrying mosquitoes), which are distinguished as follows:

Anophelini	*Culicini*
Adult —	Adult —
male palpi long, clubbed at tip.	male palpi long or short; if long, slender at tip.
female palpi as long as proboscis.	female palpi much shorter than proboscis.
Scutellum not lobed.	scutellum trilobed behind.
Egg —	Egg —
laid singly, more or less boat-shaped, with lateral air-floats.	laid singly or in rafts, elongate ellipsoidal to elongate conical, without localized floats.

Larva —
lies horizontally in the water just below the surface film and touching it at a number of points.

air-tube absent, represented only by five small flaps surrounding the tracheal openings.

Pupa —
Breathing-trumpets short and scoop-shaped, split down the front.

Larva —
hangs obliquely or vertically in the water, well below the surface film and touching it only with the tip of the air-tube.

air-tube present, usually long and well developed.

Pupa —
breathing-trumpets broadly conical to elongate tubular, not split down the front.

Tribe *Anophelini*.

For practical purposes, this Tribe includes only the genus *Anopheles*, of which many species are known to carry malaria. The genus includes a great number of closely related species, and in a publication of this size I can only include keys for the identification of the American species. Keys for the identification of adult Anopheles of various geographic regions have been published as listed below:

Europe, North Africa and Northern Asia — F. W. Edwards. A Revision of the Mosquitoes of the Palæarctic Region. Bulletin of Entomological Research, November, 1921. Vol. 12, p. 263.

Africa — F. W. Edwards. A Key for determining the African Species of Anopheles. Bulletin of Entomological Research, 1912. Vol. 3, p. 241.

India — S. R. Christophers. A Revision of the Nomenclature of Indian Anophelini. Indian Journal of Medical Research, January, 1916. Vol. 3, p. 454.

Malay Region — C. Strickland. Short Key to the Identification of the Common Anopheline Mosquitoes of the Malay Peninsula. 1915. Central Survey Office, Kuala Lumpur, Fed. Malay States.

North and Central America — Howard, Dyar and Knab. The Mosquitoes of North and Central America and the West Indies. 1917. Vol. 4, p. 966. Carnegie Institution of Washington, Publication No. 159.

America — H. G. Dyar. Notes on American Anopheles. Insecutor Inscitiæ Menstruus, July-September, 1918. Vol. 6, p. 141.

The keys for Africa, India and North and Central America mentioned above are reprinted in "Malaria at Home and Abroad" by S. P. James, 1920, John Bale Sons and Danielsson, Ltd., London.

Keys to the species of the Palaearctic, Nearctic, Neotropical, Ethiopian, Oriental and Australian Regions, mainly adapted from the keys listed above, by H. F. Carter, are to be found on pages 347 to 355 of Volume 1 of "The Practice of Medicine in the Tropics" edited by Byam and Archibald (Oxford Medical Publications, Henry Frowde and Hodder & Stoughton, The Lancet Building, 1 & 2 Bedford St., London, W. C. 2. 1921).

Key for the Identification of American Anopheline Mosquitoes

A. Key to well-defined groups of species:

1. Antennal joints with scales as well as hairs — Genus Chagasia

   Antennal joints with whorls of hairs only (Genus Anopheles) 2

2. Wing scales all dark colored—Anopheles Group, Series I
   Wings with some patches of light scales, also . . 3
3. Veins 2, 4 and 6 all dark-scaled—Dendropædium Group
   These veins with some light-scaled patches . . 4
4. Costa with light patches only at tip of first vein, or only at tips of first vein and subcosta—
   Anopheles Group, Series II
   Costa with more than two light patches 5
5. Costa with three prominent dark areas and many small accessory dark spots, each consisting of only a few scales . . . . . Arribalzagia Group
   Cost with four well-defined dark areas and with out any accessory spots 6
6. Abdomen without scales dorsally except sometimes on the last segment . Myzorhynchella Group
   Abdomen with scales on the dorsal surface of more than one segment . . Nyssorhynchus Group

B. Keys to the species included in the various groups.

Genus *Chagasia* — *C. fajardoi.*
Genus *Anopheles* —

Anopheles Group — Series I

1. Wings entirely unspotted 2
   Wings showing four dark spots, formed by aggregations of scales 5
2. Palps of female all dark, without white bands . 3
   Palps of female showing some indistinct white bands 4
3. Erect scales of back of head slender, not forked *A. nimbus*
   Erect scales of back of head expanded and forked at tip . . . . . *A. barberi*
4. Very black species; palps of female with an indistinct white band at base of next to last segment . . . . . *A. atropos*

Lighter species; palps of female with whitish bands on all segments . . . . . *A. walkeri*

5. A coppery spot on wing fringe at apex of wing *A. maculipennis.* (*occidentalis.*)
   No such coppery spot present . . *A. quadrimaculatus*

Anopheles Group — Series II

1. Costa with a light patch at tip of first vein only . 2
   Costa with light patches at tips of first vein and subcosta 3
2. Sixth vein all dark scaled . . . . . *A. eiseni*
   Sixth vein light-scaled with three dark spots *A. crucians*
3. Some of the wing scales broad and rounded — *A. grabhami*
   All of the wing scales slender and lanceolate . . 4
4. Third vein all dark, or with a white patch at tip — *A. punctipennis*
   Third vein with a large white area in the middle — *A. pseudopunctipennis*

Arribalzagia Group

1. Abdomen without lateral tufts of scales *A. vestitipennis*
   Abdomen with lateral scale tufts 2
2. All wing scales slender and lanceolate 3
   Some of wing scales broad and inflated 4
3. Hind tarsal joints with apical yellowish rings, last joint all black . . . . . *A. maculipes*
   Hind tarsal joints with numerous yellowish rings, last joint all yellow . . . . . *A. strigimacula*
   Hind tarsi with few whitish rings, last joint narrowly white at base and apex . . *A. apicimacula*
4. Hind tarsi yellowish with many small black dots . 5
   Hind tarsi blackish with whitish or yellowish rings 6
5. Palps banded with yellowish . . . *A. mediopunctatus*
   Palps not banded . . . . . *A. intermedium*

6. Only two light bands on palps, the tips black — *A. punctimacula.* (*malefactor.*)
   Three light bands on palps, the last one including the tip . . . . . *A. pseudomaculipes*

Nyssorhynchus Group

1. Hind tarsi all white beyond the second joint . . 2
   Hind tarsi white beyond second joint except for a black ring at base of last joint 4
2. Scales on dorsal surface of abdomen confined to last two segments . . . . . *A. braziliensis*
   Scales present on dorsal surface of all the abdominal segments 3
3. Lower fork of second vein with a white patch at tip . . . . . *A. argyritarsis*
   Lower fork of second vein with a black patch at tip . . . . . *A. pictipennis*
4. Palps with the last two joints mostly white *A. tarsimaculata*
   Palps with the last joint largely white, next to last joint largely black . . . . . *A. albimanus*

Myzorhynchella Group

(Last four hind tarsal joints white in all species.)

1. Last abdominal segment white-scaled dorsally *A. parvus*
   Last abdominal segment dark-scaled dorsally . 2
2. A black band at junction of second and third hind tarsal joints . . . . . *A. nigritarsis*
   No such black band present 3
3. A broad white area at junction of hind tibia and first tarsal joint . . . . . *A. gilesi*
   No such white area present . . . . . *A. lutzii*

Dendropaedium Group

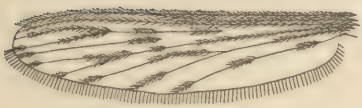
1. Abdomen with large irregular black scales dorsally *A. boliviensis*
   Abdomen without scales dorsally 2

2. Costa with white patches at tips of first vein and of subcosta only . . . . . *A. neivai*
   Costa with four white patches 3
3. Third vein black with a small white spot at base *A. hylephilus*
   Third vein broadly white in the middle 4
4. Hind tarsal joints mostly black with apical white rings . . . . . *A. bellator*
   Hind tarsal joints mostly white with basal black rings . . . . . *A. cruzi*

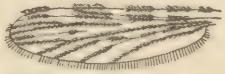
The Identification of Anopheles Larvæ

The full-grown larvæ of the different species of *Anopheles* can often be identified nearly as readily as the adults. The distinguishing characters are the form of various hairs or modified hairs borne on the head and body of the larvæ. The most important groups of these hairs are as follows:

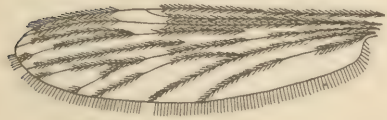
1. Clypeal hairs. — Three pairs of hairs located at the anterior end of the dorsal surface of the head. The two anterior pairs (inner and outer anterior clypeal hairs) are inserted near the edge of the head and project anteriorly. The inner pair are almost always easy to see, but the outer pair lie just over the mouth-brushes when the latter are protruded, and are sometimes hard to distinguish. Both pairs are readily observed in living larvæ at times when the mouth-brushes are drawn in and the head turned dorsal side up. When the larva is actually feeding they are entirely obscured by the movements of the mouth-brushes. The posterior pair of clypeal hairs are inserted farther back on the head and are often



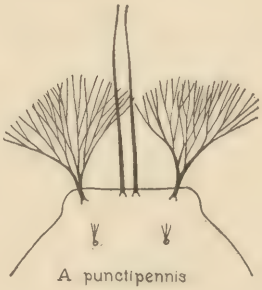
Anopheles crucians



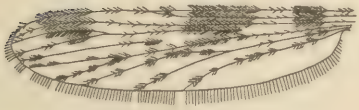
Anopheles bellator



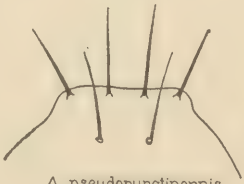
Anopheles punctipennis



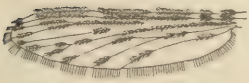
A punctipennis



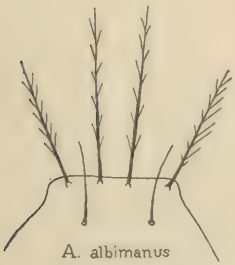
Anopheles punctimacula



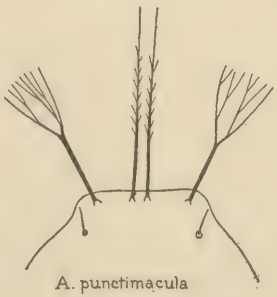
A pseudopunctipennis



Anopheles albimanus



A. albimanus



A. punctimacula

PLATE XV. — WING-PATTERN OF ADULT AND CLYPEAL HAIRS OF LARVA OF SOME AMERICAN SPECIES OF *Anopheles*.

very small and difficult to see. Fortunately they are not of importance in the identification of the larvæ. Plate XV shows some characteristic types of clypeal hairs.

2. Lateral hairs of abdominal segments 4 to 6. — In all *Anopheles* larvæ the three anterior abdominal segments bear very long and strongly feathered hairs on their sides. Segments four to six bear smaller lateral hairs, which are usually single or else split at the base into from two to six branches. In a few species whose larvæ are found in tree-holes and water held by plants, these lateral hairs are sparsely feathered.

3. Palmate hairs. — These characteristic fan-like tufts occur typically in pairs on the posterior quadrant of the thorax and on the first seven abdominal segments. In a number of species certain of these pairs of tufts are either lacking entirely or represented by very rudimentary structures which can not be seen without high magnifications. The shape of the separate leaflets of which each tuft is composed is also very characteristic for certain species or groups of species.

Further details on larval characters in *Anopheles* may be found in the following papers:

Stanton, A. T.: The Larvæ of Malayan Anopheles. Bulletin of Entomological Research. 1915. Vol. 6, p. 159.

Root, F. M.: The Larvæ of American Anopheles Mosquitoes, in Relation to Classification and Identification. American Journal of Hygiene. 1922. Vol. 2, p. 379.

Key to the Known Larvæ of American *Anopheles*

1. Outer anterior clypeal hair unbranched 2
   Outer anterior clypeal hair dichotomously branched . 5
   Outer anterior clypeal hair split up like a fan at tip
   A. fajardoi
   Outer anterior clypeal hair with fine lateral branches
   A. albimanus, A. tarsimaculata and *A. argyritarsis*
2. Lateral hairs of abdominal segments 4 to 6 feathered 3
   Lateral hairs of abdominal segments 4 to 6 not feathered 4
3. Frontal hairs (a row of six hairs between the bases of the antennæ) very short and unbranched . . . *A. barberi*
   Frontal hairs of normal length, though some or all of them are unbranched . . . . . *A. cruzi*
   leaflets of palmate hairs sharply pointed
   A. neivai
   leaflets of palmate hairs truncate at tip
4. Leaflets of palmate hairs slender, smooth, sharply pointed, not notched . . . . . *A. lutzii* and *A. parvus*
   Leaflets of palmate hairs lanceolate, notched conspicuously near the tip . . . . . *A. eiseni*
   Leaflets of palmate hairs narrowing suddenly just beyond the middle of their length and ending in a long narrow filament . . . . . *A. pseudopunctipennis*
5. Outer anterior clypeal hair with more than twenty ultimate branchlets 6
   Outer anterior clypeal hair with ten or fewer ultimate branchlets
   A. punctimacula, A. strigimacula and *A. apicimacula*
6. Inner anterior clypeal hair with fine lateral branches toward tip . . . . . *A. grabhamii*
   Inner anterior clypeal hair unbranched 7
7. Well-developed palmate hairs on abdominal segments 2 to 7
   A. punctipennis and *A. quadrimaculatus*
   Well-developed palmate hairs on abdominal segments 3 to 7
   A. crucians —
   palmate hairs on segments 3 and 7 smaller than others.
   A. maculipennis —
   all palmate hairs of about the same size.

Tribe *Culicini.*

The majority of the Culicine mosquitoes are not of real medical importance. Comparatively few species are known to be disease-carriers. The numerous genera of this Tribe can be conveniently considered as forming a number of groups, characterized particularly by the larval structure and habitat. The following tabulation includes a few of the genera of these groups which are most likely to be encountered by the medical man in the course of mosquito-survey work:

1. Sabethine group. Brilliantly colored jungle mosquitoes, whose larvæ live in water held by plants (tree-holes, bamboo joints, leaf-bases of Bromeliads, flower-bracts of Heliconias, etc.). The larvæ lack the ventral brush on the last segment of the abdomen which is found in all other mosquito larvæ. Many genera, some of which attack man, but none of medical importance.
2. Megarhinine group. Mosquitoes whose larvæ live in tree-holes and have no pecten (basal row of spines) on the air-tube. The larvæ of *Megarhinus* are large and feed on other mosquito larvæ, particularly those of *Orthopodomyia*, another member of the same group.

   Genera — *Megarhinus.* Very large, brilliantly colored mosquitoes, with a characteristic long, curved, tapering proboscis, not adapted for sucking blood.

   Orthopodomyia. Small, dark colored mosquitoes, often with pure white stripes on the thorax and white bands on the legs. The next to the last joint of the front tarsus is shorter than the last joint. For larval characters see Plate XVI.

3. Uranotænia group. Small, dark mosquitoes, with small areas of brilliant metallic-blue scales on thorax, head and

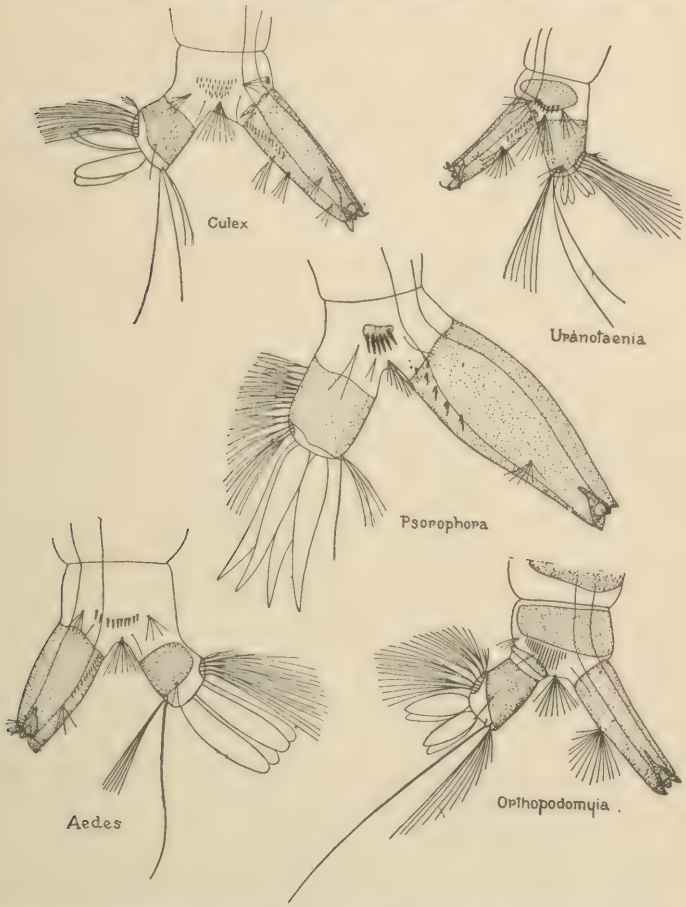


Plate XVI. — Posterior Ends of the Larvæ of Some Culicine Mosquitoes.

wings. The eggs are laid in rafts, as in *Culex*, and the larva has only one pair of hair-tufts on the air-tube, as in

Aedes (see Pl. XVI). The head of the larva is dark colored and ovoid, and the larva hangs horizontally in the water, so that it may be mistaken for a small *Anopheles* larva at first sight. Genus — *Uranotænia.*

4. *Aedine group.* The larvæ usually live in collections of water which are temporary (i.e., which dry up at some time during each year). The eggs are laid singly and the larvæ have only one pair of hair-tufts on the air-tube. The adult females in this group usually have abdomens which taper toward the tip and have two short appendages, the cerci, at the tip.

   Genera — *Psorophora.* Rather large to very large mosquitoes, with some or all of the leg scales erect. The larvæ have the single pair of hair-tufts well beyond the middle of the air-tube and some of the tufts of the ventral brush of the last segment pierce the chitinous ring which encircles this segment. The larvæ of a few species feed on mosquito larvæ, principally those of other species of *Psorophora* and of *Aedes.* (See Pl. XVI.)

   Theobaldia. Rather large mosquitoes, dull colored, with two rows of bristles just in front of the prothroacic spiracle, instead of one row, as in most other mosquitoes. The larva has the single pair of hair-tufts close to the base of the air-tube.

   Aedes. Small mosquitoes, with all the leg scales appressed. In the larvæ, the pair of hair-tufts are beyond the middle of the air-tube, and either the chitinous ring around the last segment is not complete or, if it is complete, none of the hairs of the ventral brush pierce it. (Pl. XVI.)

5. Culicine group. The larvæ usually live in permanent collections of water. The eggs are laid in rafts and the larvæ usually have four or more pairs of hair-tufts on the air-

tube. The adult females in this group usually have the abdomen broad and flattened, rather abruptly truncate at tip, with no cerci visible.

Genera — *Culex.* Small mosquitoes, usually brownish in color and without any striking markings. The larvæ have a series of hair-tufts on the air-tube.

Tæniorhynchus. Fairly small mosquitoes, usually with a "pepper-and-salt" coloration, produced by alternation of light and dark scales. The larvæ and pupæ never come to the surface of the water to breathe, but obtain air by piercing the air-holding tissues of the roots of various aquatic plants. The larval air-tube has no pecten, and only a single pair of hair-tufts. It is very short and tapers rapidly to a sharp point.

Lutzia. Large, dull colored mosquitoes, whose larvæ feed on other mosquito larvæ, mainly species of *Culex.* The larval air-tube bears a series of hair-tufts and the last abdominal segment of the larva is long and pointed.

Deinocerites. Small, blackish mosquitoes, which breed and rest in crab-holes in the tropics. The male antennæ are not bushy, but resemble those of the female. The larvæ have only a single pair of hair-tufts on the air-tube and their mandibles project laterally in an angular fashion.

The Culicine mosquitoes most important from a medical point of view are the domestic species, which breed mainly in artificial water-containers in and about houses. The three common species are listed below:

Culex pipiens — The house mosquito of temperate regions. A small brownish species which is the commonest domestic

mosquito in Europe, North Africa, Northern Asia, Argentina, and North America north of the latitude of Washington, D.C. Known to carry Filariasis in Asia.

Culex quinquefasciatus (*Culex fatigans*) — The house mosquito of the tropics. A small brownish species which is practically indistinguishable from *C. pipiens* without the services of an expert on mosquitoes. Carries Filariasis and perhaps Dengué.

Aedes (*Stegomyia*) *ægypti* (*Stegomyia fasciata*) — The yellow fever mosquito. A small black species with conspicuously white-banded legs and less obvious white thoracic stripes in a pattern which has been compared with a lyre. Very abundant in houses in most parts of the tropical and subtropical zones. Carries Yellow Fever and Dengué.

A number of other species of mosquitoes, including members of the genera *Anopheles*, *Culex*, *Aedes*, and *Tæniorhynchus* have been implicated as carriers of Filariasis. A fairly complete list is to be found in Hindle's book on "Blood-sucking Flies."

4. Family *Simuliidæ* — Genus *Simulium*.

The black-flies, buffalo-gnats or turkey-gnats are easily recognizable by their small size, dark color, short stocky build, and, of course, the characteristic structure of the antennæ and wings. The larvæ and pupæ live in running water, as a rule, attached to stones or aquatic plants. The adults are very annoying blood-suckers, but are not proved carriers of any human disease. The idea that they are concerned in the dissemination of pellagra seems to have been pretty definitely disproved. Recently Robles, in Guatemala, has suggested that two unidentified species of *Simulium* are probably the inter-

mediate hosts of a filarial worm (*Onchocerca cœcutiens*) which causes the disease known locally as Coast Erisipelas ("Erisipela de la costa").

For practical purposes, the family may be considered to include only the single genus *Simulium*.

5. Family *Tabanidæ*.

These flies are well known under the common names of horse-flies, deer-flies, mangrove flies, hippo-flies, seroots, etc. The family is sufficiently characterized by the antennæ and wing venation. It includes a great many genera and species of blood-sucking flies, the larger species usually confining their attentions to the large mammals, wild and domestic, while the smaller ones attack man as well. The larvæ live in moist soil, usually under or near water, and the pupæ occur just below the surface of the ground in drier soil, but usually in the neighborhood of water. Many of the genera are small and comparatively local in their distribution. The following tabulation includes only the large genera (genera with many species) which are of almost world-wide distribution:

1. Ocelli usually present, hind tibiæ with spurs 2
   Ocelli absent, hind tibiæ without spurs 5
2. Third segment of antenna composed of seven or eight sub-segments 3
   Third segment of antenna composed of five sub-segments 4
3. First posterior cell of wing closed before margin . *Pangonia*
   First posterior cell of wing open . . . . . *Diatomineura*

4. Second segment of antenna nearly as long as first *Chrysops*
   Second segment of antenna much shorter than first *Silvius*
5. Third antennal segment composed of four sub-segments . . . . . *Haematopota*
   Third antennal segment composed of five sub-segments . . . . . *Tabanus*

Species of *Tabanus* and *Hæmatopota* are known to be vectors of some of the diseases of domestic animals. Species of *Chrysops* serve as intermediate hosts of the human filarial worm *Loa loa* in Africa and have recently been shown to carry the bacterial disease known as deer-fly fever (Tularæmia) in the state of Utah.

6. Family *Muscidæ*.

Although the majority of the *Muscidæ* are non-blood-sucking flies like the well-known house-fly, this family also includes a compact little group of blood-sucking genera, leading by gradual stages from flies very closely allied to the house-fly up to the notorious Tsetse-flies. Most of these species of blood-sucking *Muscidæ* inhabit Africa and the Orient. Certain species of the genera *Stomoxys* and *Lyperosia* have been carried all over the world with the domestic animals which constitute their main source of food. The blood-sucking genera may be identified by the following key:

1. Arista (bristle on third segment of antenna) feathered on both dorsal and ventral sides (as in Pl. XII, *Musca*) 2
   Antennal arista feathered on dorsal side only 5

2. Mouth parts fleshy at tip, like those of a house-fly *Philæmatomyia*
   Mouth parts form a slender chitinized proboscis . . . 3
3. First posterior cell of wing much narrowed at wing margin . . . . . *Hæmatobosca*
   First posterior cell widely open at wing margin . . . 4
4. Third vein of wing with a few bristles near tip . *Hæmatobia*
   Third longitudinal vein without any bristles *Bdellolarynx*
5. Individual hairs of arista plumose (Pl. XII, *Glossina*) *Glossina*
   Individual hairs of antennal arista not plumose (Pl. XII, *Stomoxys*) 6
6. First posterior cell of wing much narrowed at margin *Stygeromyia*
   First posterior cell widely open at wing margin . . . 7
7. Palpi slender, half as long as proboscis . . . . *Stomoxys*
   Palpi spatulate, nearly as long as proboscis . . . *Lyperosia*

The majority of these flies lay their eggs in cow-dung or other decaying vegetable matter, and their larvæ or maggots greatly resemble those of the house fly. In the genus *Glossina* (Tsetse-flies), however, the egg hatches within the body of the female fly and the maggot is retained in the maternal uterus until it is full grown and ready to pupate, when it is expelled. Apparently the female fly carefully selects a suitable place for the larva to pupate before dropping it.

Most of these blood-sucking *Muscidæ* are not known to carry any human diseases, although some of them are implicated in the spread of diseases of domestic animals. An exception must be made in the case of the notorious Tsetse-flies (genus *Glossina*), which are found only in the Ethiopian Region. Prac-

tically all of the species of this genus are known to carry trypanosomes which cause diseases of domestic animals. The human diseases known as trypanosome fevers in the early stages and as Sleeping Sickness in later ones, are carried by the following species:

Gambian Sleeping Sickness — caused by *Trypanosoma gambiense* — carried by *Glossina palpalis*.

Rhodesian Sleeping Sickness — caused by *Trypanosma rhodesiense* — carried by *Glossina morsitans*.

According to recent and unconfirmed reports, we must add also:

Nigerian Sleeping Sickness — caused by *Trypanosma nigeriense* — carried by *Glossina tachinoides*.

For further information regarding *Glossina*, Austen's "Handbook of the Tsetse-flies" (British Museum, 1911) or Austen and Hegh's "Tsetse-flies" (Imperial Bureau of Entomology, London, 1922), as well as the numerous articles in the *Bulletin of Entomological Research*, should be consulted.

7. Family *Hippoboscidæ*.

This family includes a number of blood-sucking flies which differ from other *Diptera* in being ectoparasites of birds and mammals during the adult stage of their lives. Like the Tsetse-flies, the female retains the larva within her uterus until it is ready to pupate. Since these flies rarely attack man, they are not of great medical importance. The most familiar member of this group is the wingless species

usually called the "sheep-tick" or "ked" (*Melophagus ovinus*). Winged species of the genus *Hippobosca* are parasitic on horses, cattle, dogs, etc., in the warmer regions of the Old World, and there are numerous genera which parasitize birds, particularly herons and birds of prey, in all countries.

Some very peculiar allied forms are parasitic on bats only ("spidery" flies of the family *Nycteribiidæ*, and minute, winged flies of the family *Streblidæ*).

2. *Filth Flies.*

The typical example of this group is the House-fly (*Musca domestica*). The flies which frequent houses, markets, bazaars, etc., are practically all species which feed both on human excrement and on human food. One group, typified by the house-fly, also breeds in excrementitious matter and resorts to human feces both for feeding and for oviposition. Another group, including the blow-flies and blue-bottles, breeds in decaying meat as a rule, and visits human excrement only to feed on it. Both groups are evidently in a position to transport disease-producing organisms from human feces to human food. Just how great an effect such transport has on the incidence of the various intestinal diseases is still a mooted question. The diseases which such flies are accused of carrying include typhoid and paratyphoid fevers, cholera, dysentery (both bacillary and protozoan) and infantile diarrhea.

The following outline may be of assistance in

determining the genera to which flies commonly found in and around houses belong:

A. Fourth longitudinal vein of wing bent at a sharp angle shortly before it reaches the margin of the wing.

1. Body coloration not metallic.

a. Antennal arista plumose to the tip.

Musca — Thorax longitudinally striped with black and gray. No small woolly hairs on the thorax. Very common in houses, especially in warm weather. Larvæ in horse manure and other fecal matter.

Pollenia — Thorax all dark, not striped. Many small woolly yellow hairs among the thoracic bristles. Adults hibernate in houses from fall to spring, but are not usually present in the summer. Larvæ parasitic in earthworms.

See also *Philæmatomyia* under blood-sucking *Muscidæ*.

b. Antennal arista plumose only for about two-thirds of distance to tip.

Sarcophaga — Gray flies with black stripes on the thorax and checkered abdomens. Sometimes found in houses. Larvæ in decaying meat, fecal matter and parasitic in various other insects.

2. General body coloration metallic.

a. Color of thorax and abdomen dark blue or blackish.

Calliphora — Dorsal thoracic bristles behind the transverse suture well developed. Forceps of male genitalia small and inconspicuous. Abdomen dull blue with a watered appearance. Common in houses. Larvæ in decaying meat.

Cynomyia — Dorsal thoracic bristles behind the transverse suture well developed. Forceps of male genitalia long and prominent. Abdomen bright metallic blue or greenish throughout. Common in houses. Larvæ in decaying meat.

Phormia — Dorsal thoracic bristles behind the transverse suture poorly developed. Body color blackish rather than blue. Common in houses. Larvæ in decaying meat.

b. Color of thorax and abdomen bright green, sometimes with a bluish or bronzy lustre.

Lucilia — Cheeks gray or silvery. Sternopleural bristles two in front and one behind. Thorax without stripes. Common in houses. Larvæ in decaying meat.

Cochliomyia (formerly called *Chrysomyia*) — Cheeks buff or reddish. Sternopleural bristles two in front and one behind. Thorax with dark longitudinal stripes. Rather rare in houses. American only. Larvæ in decaying meat or living animals ("screw-worms").

Chrysomyia (formerly called *Pycnosoma*) — Cheeks buff or reddish. Sternopleural bristles one in front and one behind. Thorax without stripes. Old World only. Larvæ in decaying meat or living animals.

B. Fourth longitudinal vein of wing straight or gently curved as it approaches the margin of the wing.

1. Fourth longitudinal vein straight near wing margin.

Fannia — Decidedly smaller than house-flies, but with same type of coloration. Common in houses. Larvæ in excrement, garbage, etc.

Ophyra — About the same size as *Fannia*, but color shining black. Not common in houses. Larvæ in garbage, meat, living animals, etc.

2. Fourth longitudinal vein curved near wing margin.
Muscina — Slightly larger than house-flies, but with same type of coloration. Common in houses. Larvæ in decaying vegetable matter, including fecal matter.
See also *Stomoxys* and *Lyperosia* under blood-sucking *Muscidæ*.

The genera tabulated above are all flies of fair size, none being included which are much smaller than the common house-fly. A number of genera of extremely small flies also occur in houses. The two commonest of these are probably the following:

Drosophila — General color brownish or yellowish. Abdomen usually banded with black. Particularly attracted by bananas and other fruit. Larvæ in decaying fruit, etc.

Piophila — General color shining black, abdomen not banded. Larvæ in old cheese, etc. ("cheese-skippers").

3. *Myiasis-producing Flies.*

The flies whose larvæ may be found living as parasites in the bodies of man and other mammals belong to a number of different groups. The various types of parasitism by fly larvæ may be summarized as follows:

A. Bot and Warble Flies (family *Œstridæ*). — Rather large flies whose larvæ can develop only as parasites in the bodies of mammals. The larvæ are usually rather short and fat, not tapering to a point at the anterior end, as most muscoid larvæ do. The larvæ may inhabit (1) the alimentary canal or (2) the nasopharynx of their host or (3) may take up a position just below the skin, forming sores which somewhat resemble a boil. The larvæ seem to feed

on exudations, without any extensive destruction of the host tissues. A few species have been found in man, but the great majority occur only in lower mammals.

B. Flies of the blow-fly (Family *Muscidæ*, Subfamily *Calliphorinæ*) or flesh-fly (Family *Sarcophagidæ*) groups — Smaller flies, with larvæ of the normal muscoid type. The majority of the species breed in decaying meat, but occasionally larvæ may be found in neglected wounds, etc. A few species, while retaining the ordinary larval habitat to a considerable extent, occur frequently in neglected wounds, in the soiled wool of sheep, and in other similar places (examples, *Cochliomyia macellaria*, the "screw-worm," in America and *Chrysomyia albiceps* and other "wool-maggots" in Australia). And a few species seem to have given up the habit of breeding in decaying meat entirely and now depend solely on neglected wounds in animals and man (examples, *Chrysomyia bezziana* in India and probably species of the Sarcophagid genus *Wohlfahrtia* in both Europe and America). In all cases of parasitism by blow-fly and flesh-fly larvæ, rather extensive destruction of the host tissues is the rule.

C. Larvæ of flies of many different families (*Muscidæ*, *Anthomyidæ*, *Sarcophagidæ*, *Sepsidæ*, *Syrphidæ*, and others) are occasionally found in the human alimentary canal, doubtless being ingested by accident. Only rarely are symptoms produced by this "parasitism." Larvæ of this group may be voided alive, either per os or per anus.

D. Blood-sucking Maggots. — A small number of flies, mostly belonging to the families *Muscidæ* and *Anthomyidæ*, have larvæ which obtain food by sucking the blood of birds, mammals or man. These, of course, have the same potentiality for disease-transmission as any other blood-sucking insect. The only such larva which attacks man is the "Congo Floor-maggot" (the larva of *Auchmeromyia luteola*), which hides during the day in the cracks of the mud floors of native huts and crawls out at night to suck the blood of the owners. It is found only in Africa.

In most cases of Myiasis, it is difficult for the medical man to rear the larvæ to adults in order to identify the species concerned. If third-stage (nearly mature) larvæ are found, they can usually be identified as belonging to a given group of genera, at least, by an examination of the posterior spiracles, a pair of dark colored plates situated on the truncated posterior end of the larva. The general appearance of some of these spiracles is shown on Plate XVII. Each member of the pair usually consists of a chitinous "ring," completely or almost completely encircling the whole structure, a small, solid or perforated, chitinous region called the "button," which may form a part of the ring (Pl. XVII, *Calliphora*), may lie within the ring (Pl. XVII, Musca), or may be entirely absent (Pl. XVII, Phormia), and three straight or sinuous "slits" lying within the ring. These slits are the apertures through which the larva breathes, and they are divided up into many small apertures by a series of chitinous bars

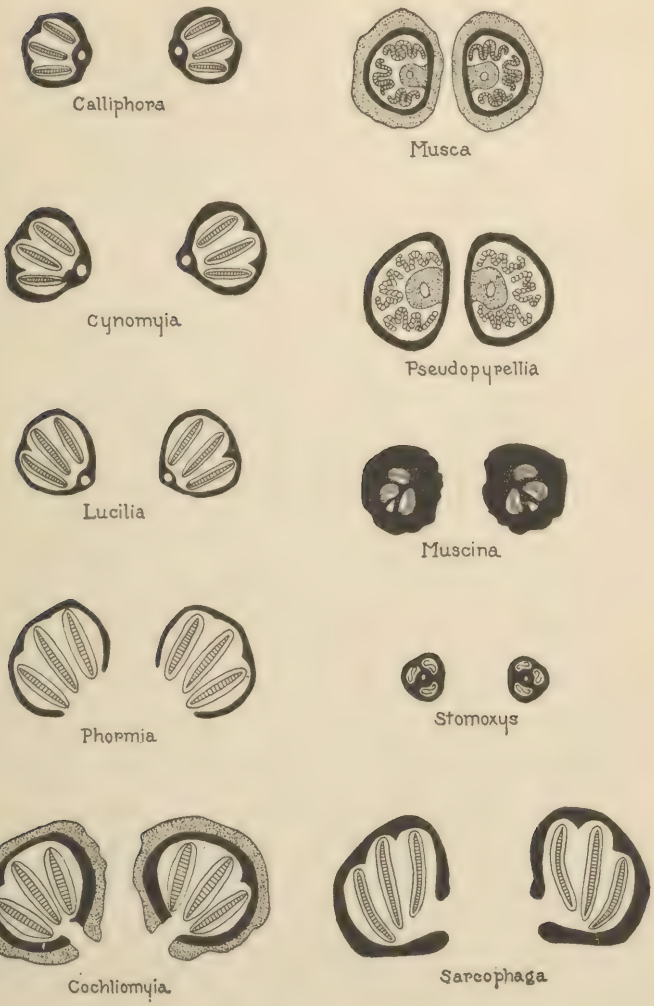


PLATE XVII. — POSTERIOR LARVAL SPIRACLES OF SOME COMMON MUSCOID FLIES.

which cross them. These structures are outlined with great clearness and delicacy in the young third-stage larvæ, but in some species they become so heavily chitinized as the time for pupation approaches that only the slits can be made out, showing dimly through the apparently solid spiracular plate. Usually, however, the original conditions can be made out enough to permit of identification.

The following tabulation includes the genera whose larvæ are most often concerned in human Myiasis, as well as other genera of muscoid flies which a sanitarian might wish to identify in connection with an anti-fly campaign. Where genera are listed in two columns, the left-hand column includes the common and important genera, while in the right-hand column are given the names of other genera of the same group which are so rare or local in their breeding places that they are not likely to come to the notice of any but an intensive student.

A. Larvæ of the normal muscoid shape, slender, truncate posteriorly, tapering anteriorly.

1. Larvæ with lateral fleshy processes.

Fannia — posterior spiracles at tips of short tubercles.

Chrysomyia (*Pycnosoma*) — (species *albiceps*, *varipes*, and *villeneuvii* only) — posterior spiracles on truncate end of abdomen, as usual.

2. Larvæ smooth, without lateral processes.

a. Posterior spiracles D-shaped, button area within ring, slits thrown into loops.

Musca *Pyrellia.*
Philæmatomyia. *Pseudopyrellia.*
Lyperosia. *Morellia,* etc.

b. Posterior spiracles triangular with rounded corners, button area within ring, slits S-shaped.
Stomoxys. *Bdellolarynx.*
Hæmatobosca.

c. Posterior spiracles nearly circular, button area within ring (often obscured by chitinization), slits only slightly bent.
Muscina.

d. Posterior spiracles nearly circular, button area a part of ring, slits nearly straight.
Calliphora.
Cynomyia.
Lucilia.

e. Posterior spiracles more or less circular, button area absent, leaving ring incomplete, slits nearly straight.
1. Slits all sloping downward and inward.
Chrysomyia (except species mentioned above).
Cochliomyia.
Phormia.
2. Inner slits sloping downward and outward, middle slits nearly vertical, outer slits sloping downward and inward.
Sarcophaga.
Wohlfahrtia.

B. Larvæ not of the normal muscoid shape.
1. Larvæ short and stout, not much tapered at either end. (*Œstridæ.*)
a. Posterior spiracles opening by three slits on each side.
Gastrophilus — spiracles partly covered by folds of body.

Dermatobia — spiracles on end of last segment, which can be telescoped within next to last segment.

b. Posterior spiracles opening by a large number of scattered round or oval openings.

Œstrus — Button area in center of plate.

Hypoderma — Button area forming a deep insert in inner side of the kidney-shaped plate.

2. Larvæ short and stout, bearing at posterior end a long, slender tube, as long as or longer than the rest of the body ("rat-tailed maggots").

Eristalis (*Syrphidæ*).

B. *Order Hemiptera — True Bugs*

The majority of the *Hemiptera* (here understood as not including the homopterous forms) can be at once distinguished from all other insects by their wings, which have the basal half leathery and the apical half membranous in the fore wing, while the hind wing is membranous throughout. Some species, however, including some of medical importance, have the wings very much reduced, in fact nearly absent. These may be recognized as *Hemiptera* by other characteristics, particularly by the fact that the mouth parts form a rather long, jointed beak, arising at the anterior end of the head and bent down and backward so that it lies along the ventral surface of the head and thorax.

Only two Families of this Order are of real significance to the medical man. Both belong to the Suborder *Gymnocerata*, including mainly terrestrial forms with long and conspicuous antennæ. They

are distinguished from other Families by the following combinations of characters:

Family Cimicidæ — Hind coxæ hinged, clypeus triangular, ocelli absent, wings much reduced, antennæ four-jointed, beak three-jointed, tarsi three-jointed.

Family Reduviidæ — Hind coxæ with a ball-and-socket joint, ocelli present, wings well developed, antennæ four-jointed and slender throughout, beak three jointed, tarsi three-jointed, claws apical.

Family *Cimicidæ* — Bed-bugs.

These are rather small, flat, mahogany-colored bugs which lie hidden in crevices during the day and come out to feed on blood by night. Their hind wings are absent and the fore wings are reduced to small, scale-like structures. Most of the species belong to the genus *Cimex*, in which the anterior angles of the first thoracic segment project more or less on either side of the head and the scutellum is triangular. The two species which habitually feed on human beings have habits which would enable them to carry blood-parasites from man to man, and they are under suspicion in the cases of Kala-Azar and Oriental Sore.

Genus *Cimex* —

Cimex lectularius — the human bed-bug of temperate climates — Anterior angles of pronotum (first thoracic segment) decidedly projecting, sides of pronotum flat and thin.

Cimex hemiptera — the human bed-bug of the tropics — Anterior angles of pronotum only slightly projecting, sides of pronotum not flattened, abdomen more elongate oval than in *C. lectularius*.

Other species of the genus attack birds and other lower animals and are to be looked for in their nests or haunts. They will usually bite human beings, but only rarely have the opportunity. The following species may be mentioned:

Attacking poultry — *C. inodorus* — (Tropical America).
Attacking pigeons — *C. columbarius* — (Europe).
Attacking swallows — *C. hirundinis* — (Europe).
C. vicarius — (America).
Attacking bats — *C. pipistrelli* — (Europe).
C. pilosellus — (America).

Family *Reduviidæ* — Assassin-bugs.

Most of the assassin-bugs are predaceous, feeding on other insects by piercing their bodies and sucking out the juices. A few genera have acquired the blood-sucking habit, at first feeding on various small wild mammals and living in their nests or burrows, and then, in the case of some species, transferring their affections to man when the opportunity arose. These last species now have almost the same habits as bed-bugs, with an even greater range of attack because of the powers of flight of the adult stage. Species of the genera *Triatoma* and *Rhodnius* are known to carry the trypanosome which causes Chagas' Fever in South and Central America and another species of *Triatoma* (*T. rubrofasciata*), which is found in tropical littorals all over the world, has been suggested as a possible vector of Kala-Azar in India.

These genera differ from the others of the family as follows:

Ocelli present, anterior coxae short, ocelli posterior to the compound eyes, thorax constricted at middle or anterior

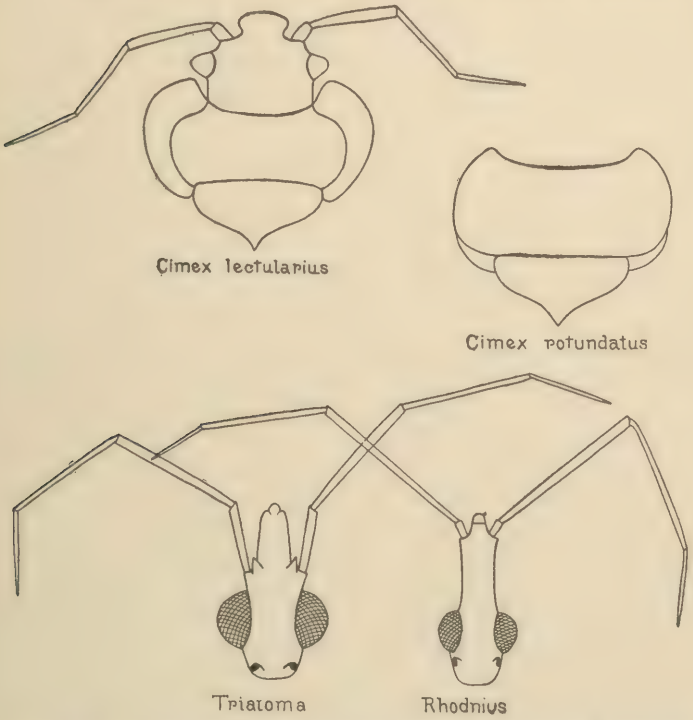


PLATE XVIII. — HEAD AND THORAX OF *Cimex lectularius*, THORAX OF *C. hemiptera* (*rotundatus*), AND HEADS OF *Triatoma* AND *Rhodnius*.

to middle, anterior tarsi three-jointed, apex of scutellum narrow, tipped with a single spine, ocelli at least as far apart as compound eyes, antennæ inserted laterally, head produced anteriorly —

Rhodnius — antennæ inserted near anterior end of head (Pl. XVIII).

Triatoma — antennæ inserted far from anterior end of head (Pl. XVIII), eyes large, body not hairy.

Meccus — antennæ inserted far from anterior end of head, eyes small, body slightly hairy. (A species of this genus has also been reported as attacking man in Costa Rica.)

Genus *Rhodnius* — *R. prolixus* (West Indies and Venezuela), is known to carry Chagas' Fever.

Genus *Triatoma* — According to a review of the genus by Neiva, about 36 species are known, 32 occurring in tropical and subtropical America, 2 in Africa, 1 in the East Indies and 1 (*T. rubrofasciata*) is found in littoral regions all through the tropics. Neiva states that all the species whose habits are known frequent either houses or the nests or burrows of small mammals for the purpose of obtaining blood.

The following species are known to carry Chagas' Fever in Brazil:

T. chagasi, *T. infestans*, *T. megista*, and *T. sordida*.

C. *Order Siphonaptera* — Fleas

Fleas are probably well enough known so that there is no need to describe them in detail. Their winglessness, jumping ability, and laterally flattened form are sufficient to distinguish them from all other insects. The species which are of interest to the sanitarian include the common fleas of man and some of the domestic animals, the fleas commonly found on rats, mice and a few wild rodents (on account of their importance as vectors of Bubonic Plague) and the chigoe or burrowing flea (genus *Tunga*) of the tropics. The genera to which these

species belong can be readily separated by the characters given in the following synopsis. If female fleas are examined, cleared in caustic potash and mounted in balsam, the shape of the conspicuous seminal receptacle, which lies within the abdomen, usually near the posterior end, is of great value for both generic and specific determination. See Plate XIX, for illustrations of the characteristics of various fleas.

A. Thoracic segments short, the three together no longer than the first abdominal segment.

Echidnophaga — a patch of spines on inner side of basal joint (coxa) of hind leg.

Tunga (*Dermatophilus*) — no patch of small spines in this position.

B. Thoracic segments longer, the three together decidedly longer than the first abdominal segment.

1. No combs on either head or thorax.

Pulex — plate to which middle leg is attached has no vertical rod-like thickening.

Xenopsylla — this plate has a vertical rod-like thickening.

2. Comb present on thorax, but not on head.

Ceratophyllus — antennal club segmented all around.

Hoplopsyllus — antennal club segmented only on posterior side.

3. Combs present on both head and thorax.

Ctenocephalus — eyes present.

Neopsylla — eyes absent.

The following list includes the species most likely to be encountered in medical or sanitary work:

Echidnophaga gallinacea — common on fowls, occasional on rats.

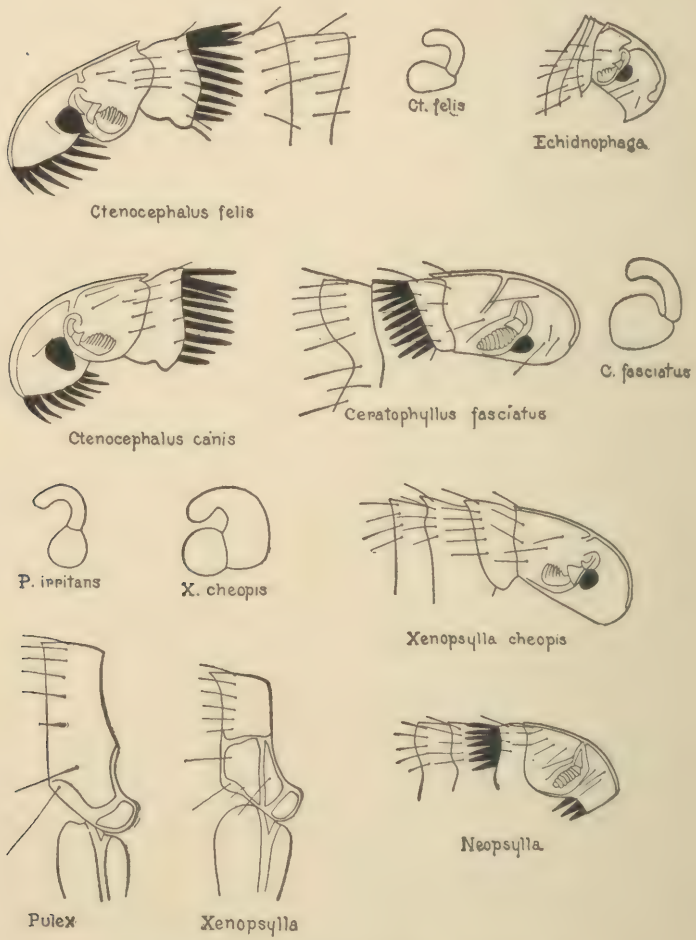


PLATE XIX. — DISTINGUISHING CHARACTERS OF VARIOUS FLEAS.

Head and thorax of *Ct. felis* and *canis*, *Xenopsylla*, *Ceratophyllus*, *Echidnophaga* and *Neopsylla*; seminal receptacles of female of *Ctenocephalus*, *Ceratophyllus*, *Pulex* and *Xenopsylla*; middle thoracic segment and base of middle leg of *Pulex* and *Xenopsylla*.

Tunga (formerly called *Dermatophilus*) *penetrans* — the Chigoe or burrowing flea. Pregnant females burrow into the skin of man, pigs and other animals in tropical America and Africa, causing considerable annoyance.

Pulex irritans — the human flea. Common on man in Europe, the West Indies, the Pacific coast of the United States, and other regions. Found commonly on pigs in the Atlantic coast region of the United States, but very rare as a human pest in this region.

Xenopsylla cheopis — the tropical rat-flea. Probably the species most often concerned in the transmission of Bubonic Plague.

Xenopsylla astia — another rat-flea, common in some parts of the Orient. Probably not a good vector of Plague.

Ceratophyllus fasciatus — the rat-flea of temperate regions. Also known to transmit Bubonic Plague.

Ceratophyllus acutus — The predominant flea of the California Ground-Squirrel. Able to transmit Plague.

Hoplopsyllus anomalus — another common Ground-Squirrel flea which is able to transmit Plague.

Ctenocephalus felis — the cat-flea.

Ctenocephalus canis — the dog-flea. These two species are the ones usually concerned in house infestations in the eastern United States. *Ct. canis* has also been accused of carrying Infantile Kala-Azar in the Mediterranean region.

Neopsylla musculi — the mouse-flea. Probably does not transmit Plague, since it is not known to bite man.

D. *Order Siphunculata — Lice*

The human lice are probably well known (though not necessarily from personal experience) to every medical man. There are many other species of lice on wild and domestic animals, but they are so delicately adjusted to their particular host species that they do not form a medical problem.

The lice of man and the monkeys belong to a

special Family, the *Pediculidæ*, characterized by the presence of large, presumably functional eyes. It contains the following genera:

Pediculus — antennæ five-jointed in the adult, legs all of about the same size.

The head and body lice of man are now considered to constitute a single species, *Pediculus humanus*, with two varieties or sub-species, *P. humanus capitis* and *P. h. corporis*. Recent experiments have proved that they are vectors of Relapsing Fever, Typhus, Trench Fever, and other diseases.

Other species of *Pediculus* occur on the Old World anthropoid apes and the *Ateles* apes of tropical America.

Phthirius — antennæ five-jointed in the adult, front pair of legs smaller and weaker than the others.

The only species is *Phthirius pubis*, the crab-louse of man. This species has not as yet been incriminated as a disease carrier.

Pedecinus — antennæ three-jointed even in the adult stage, legs all about the same size.

This genus includes the characteristic lice of the monkeys.

Phthirpedecinus — antennæ three-jointed in the adult, front pair of legs smaller and weaker than the others.

A single species, found on monkeys, having the same general relationship to *Pedecinus* that *Phthirius* does to *Pediculus*.

3. Class Arachnida

The *Arachnida*, including the spiders and their numerous relatives, are grouped into eleven Orders, of which only three are of any medical interest. These may be characterized as follows:

Order *Acarina* — Mites and Ticks — Body an unsegmented sac, to which is attached a movable capitulum or "false head" bearing the mouth parts.

Order *Araneida* — Spiders — Body divided into a hard cephalo-thorax (head and thorax fused) and a soft bag-like abdomen. The second pair of appendages of the cephalo-thorax are palplike (i.e., they resemble a very small pair of legs).

Order *Scorpionidea* — Scorpions — Body divided into cephalo-thorax and abdomen, both hard, the abdomen segmented. The last five abdominal segments are drawn out into a slender tail, bearing a curved poison-spine connected with poison glands. The second pair of appendages of the cephalo-thorax are large prehensile organs resembling the claws of a lobster.

A. *Order Acarina*

Mites, as the members of this Order are usually called, are commonly of very small size. One Family, the *Ixodidæ*, includes only fair-sized species and these have been distinguished by the special name of Ticks. Several Families of mites are of great importance to medical men and others acquire a slight medical interest because a few of their species may parasitize man, either habitually or by accident.

The distinctions between the different Families often involve differences in the structure of the mouth parts, which in this Order are mounted on a movable base, the capitulum or false head. The mouth parts consist of two pairs of true appendages and a single median unpaired structure, the hypostome. The hypostome lies ventrally, while the two paired appendages are dorsal. The inner pair (cheliceræ) are prehensile or piercing organs, often armed with teeth or ending in pincers. The outer

pair (pedipalps) are made up of several joints and look like a very small pair of legs.

In the mites the true legs are usually eight in number, as in all the *Arachnida*, but number only six in the first-stage larva. They may terminate in claws, in long bristles, or in slender suckers. The Families of medical interest are included in the following synopsis:

A. Body slender, vermiform and annulated — *Demodecidæ*.
B. Body sac-like, not annulated.
  1. One pair of spiracles present, located behind the basal joint of the third or fourth pair of legs.
    Ixodidae — cheliceræ ending in a series of hook-like teeth, hypostome with numerous teeth.
    Gamasidae — cheliceræ ending in pincers, hypostome without teeth.
  2. No spiracles visible, at least in posterior part of body.
    a. Pedipalps of four or five joints, legs usually ending in claws, never in suckers — *Trombidiidæ*.
    b. Pedipalps of three joints, two anterior and two posterior pairs of legs widely separated, some of the legs ending in suckers.
      a′. Pedipalps united to head, cheliceræ not ending in pincers, females with a pair of club-shaped bristles just behind bases of anterior pair of legs — *Tarsonemidæ*.
      b′. Pedipalps free, cheliceræ ending in pincers, female without club-shaped bristles.
        Sarcoptidæ — Integument striated.
        Tyroglyphidæ — Integument not striated.

1. Family *Demodecidæ* — Follicle Mites.

This Family contains the single genus *Demodex*. The human follicle mite, *Demodex folliculorum*, is

said to occur in the hair follicles and sweat glands of a large proportion of human beings. Ordinarily, at least, no harm results from its presence. Other species are found in lower mammals, one form found in the dog giving rise to a deep-seated and refractory type of mange.

2. Family *Ixodidæ* — Ticks.

The members of this group are of large size for mites and are all blood-sucking parasites of man and other vertebrates. Certain species are known to be concerned in the transmission of at least two human diseases, and others carry a number of serious maladies of domestic animals.

Two subfamilies may be recognized:

Argatinæ — Whole body soft and leathery, without a dorsal chitinized shield or scutum. Capitulum ventral in position in adult.

Ixodinæ — Body with a hard chitinized shield or scutum dorsally. Capitulum anterior in position in the adult.

A. Subfamily *Argatinæ* — Soft Ticks.

The Soft Ticks, in general, resemble bed-bugs in their habits, hiding in crevices during the day and creeping out at night to feed. Usually each individual feeds more than once in each of its three stages (larva, nymph and adult), either on the same or on different hosts, as chance decides. There are only two genera:

Argas — Body flat, with a thin sharp striated margin when tick is unfed. Integument marked with radiating lines of disks.

Ornithodorus — Body rather rotund, with thick margin, even in unfed tick. Integument mammillated or spiny.

The species of medical importance are listed below:

Argas persicus — Fowl Tick — (cosmopolitan in sub-tropics) — carries fowl spirochaetosis. Bite said to be serious for man.

Argas reflexus — Pigeon Tick — (Europe and North Africa) — bite said to cause much local inflammation in man.

Ornithodorus moubata — (Tropical Africa) — carries Relapsing Fever.

Ornithodorus savignyi — (Africa, India and the Near East) — said to carry Relapsing Fever in Abyssinia.

Ornithodorus talaje — (Tropical America) — carries Relapsing Fever in Panama.

Ornithodorus turicata — (Tropical America) — said to carry Relapsing Fever in Colombia.

Ornithodorus megnini — The Spinose Ear Tick — (Mexico and Southern and Western United States) — parasitizes the ears of domestic animals and occasionally of man.

B. Subfamily *Ixodinæ* — Hard Ticks.

The most characteristic feature of the Hard Ticks, the dorsal shield or scutum, exhibits marked sexual dimorphism. In the male, the scutum almost covers the entire body, while in the female and the early stages it is reduced to a small plate just behind the capitulum. For this reason, perhaps, the female is much more distensible than the male, and may swell up to the size of a filbert when engorged.

Each individual Hard Tick usually attaches itself to three hosts during the course of its life-history, one in each stage (larva, nymph, adult), remaining

attached to the host for several days and then dropping off, gorged with blood, to digest the meal and either transform to the next stage or oviposit.

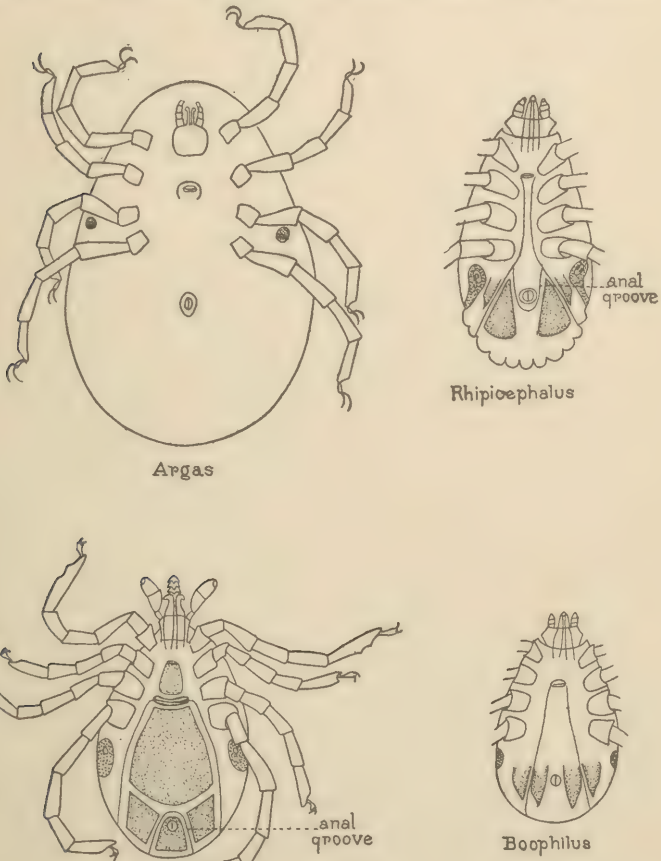


PLATE XX. — VENTRAL VIEW OF *Argas* AND OF MALES OF *Ixodes*, *Boophilus*, AND *Rhipicephalus*.

A few species have evolved a short cut by remaining attached to the host during one or both of the transformations or molts. In the genus *Boophilus*, for example, only a single host is required for the entire life-history. Once the larva of *Boophilus* attaches to a host, the completion of its life-cycle is assured, for it remains firmly attached to this same host individual during the three feeds and two molts which occur before it drops off as an engorged adult.

The easy identification of ticks depends very largely on finding the small and inconspicuous males, since the engorged females of all ticks look very much alike. The males often have hard shields of various shapes ventrally, in addition to the dorsal scutum, and these are very characteristic for the different genera. Other characters of importance are furnished by the groove which partially encircles the anus (anal groove), and the structure of the capitulum. The various species of a genus are usually very similar and can be determined only by a specialist.

The following synopsis includes all the genera of the Hard Ticks:

A. Anal groove encircling the anus anteriorly — *Ixodes*.
B. Anal groove encircling the anus posteriorly or absent.
    1. Bases of pedipalps projecting laterally beyond the base of the capitulum — *Hæmaphysalis*.
    2. Bases of pedipalps not projecting laterally.
        a. Mouth parts about the same length as base of capitulum.

a′. Male without ventral shields.

Dermacentor — base of capitulum rectangular in dorsal view.

Rhipicentor — base of capitulum hexagonal in dorsal view.

b′. Male with ventral shields.

Rhipicephalus — anal groove present.

Margaropus — anal groove absent, male with a single ventral shield anterior to anus, ending posteriorly in two points.

Boophilus — anal groove absent, male with four ventral shields, two on each side of anus.

b. Mouth parts much longer than base of capitulum.

a′. Male with ventral shields — *Hyalomma*.

b′. Male without ventral shields.

Amblyomma — Eyes present, body oval.

Aponomma — Eyes absent, body nearly circular.

Various species of Hard Ticks are of great importance to veterinarians, since they act as vectors of the entire group of diseases of domestic animals caused by *Piroplasma* and related parasites. Only a few species are of medical importance. Rocky Mountain Spotted Fever, a typhus-like disease confined to a small area in the northwestern United States, is transmitted by *Dermacentor andersoni* (*venustus*). Tick Paralysis, resulting from the bites of *Dermacentor andersoni* in the United States and Canada, and from the bites of species of the genus *Ixodes* in Africa and Australia, is probably caused by the outpouring of poisonous salivary secretions as the ticks engorge and not by any parasitic micro-organism.

3. Family *Gamasidæ* — Tick-mites.

These mites have a considerable resemblance to miniature Ticks, but may be distinguished by the

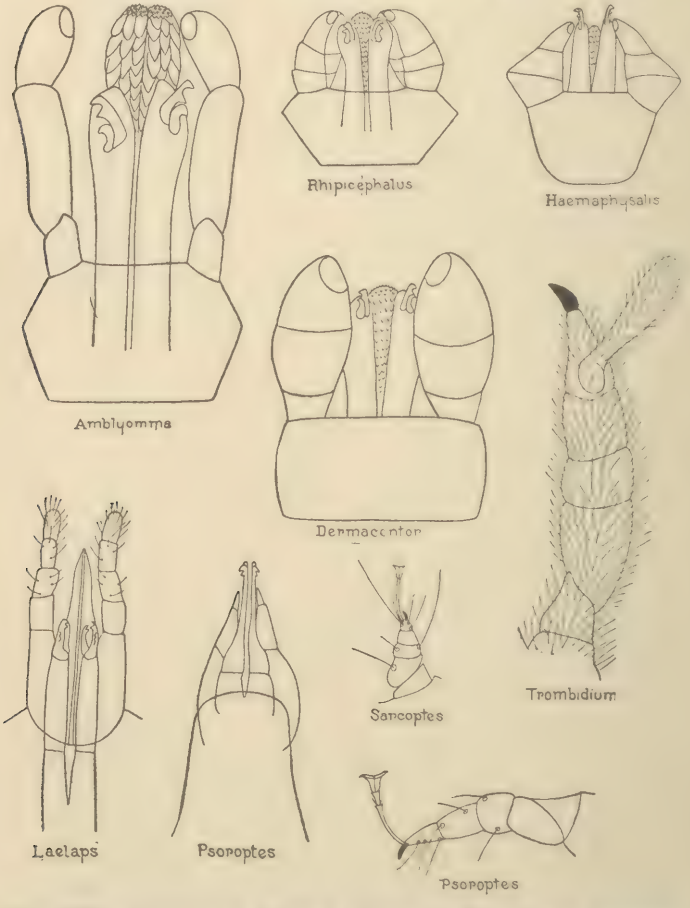


PLATE XXI. — CAPITULUM OF *Amblyomma*, *Rhipicephalus*, *Haemaphysalis*, *Dermacentor*, *Laelaps* AND *Psoroptes*; PEDIPALP OF *Trombidium*; LEG OF *Sarcoptes* AND *Psoroptes*.

cheliceræ, which end in pincers and are capable of great protrusion. Some species are predaceous, others are parasitic on insects, and a few are parasites of birds and mammals. The species which normally occur on other vertebrates may attack man by accident. The species most likely to be concerned in such attacks are *Dermanyssus gallinæ*, a common parasite of fowls, and the species of the genus *Lælaps*, which are often found in great numbers on rats and in their nests.

4. Family *Trombidiidæ* — Velvet Mites.

The adults are called Velvet Mites because of their red color and extreme hairiness. The larvæ are even more notorious under the names of Harvest-mites, "Red-bugs" or "Chiggers." Besides the characters mentioned in the table, it may be noted that in the nymphs and adults the last joint of the pedipalps bears a large club laterally. The adults are either predaceous or plant-feeders and do not attack man. The six-legged larvæ of a number of species are normally parasitic on insects or on small mammals and attack man readily, producing red inflamed spots which itch intolerably. In the Orient the larvæ of a number of species of the genus *Trombicula* are found on field mice and one species, identified as *Trombicula coarctata*, transmits to man the disease known as Tsutsugamushi, or Japanese River Fever (Flood Fever). The adults of the genus *Trombicula* are distinguished by a strong constriction of their

bodies, giving them the appearance of a bag with a string tied around it.

5. Family *Tarsonemidæ* — Louse-mites.

This family includes small, soft-bodied mites, most of which are parasites of plants or insects. Species belonging to either of these groups may accidentally attack man, producing pappular eruptions which itch persistently. The most notorious species is *Pediculoides ventricosus*, normally parasitic on insects, particularly various straw-moths. The opportunity for attacking man occurs during threshing or when persons sleep on mattresses filled with untreated straw.

6. Family *Sarcoptidæ* — Itch and Mange Mites.

These minute mites are all parasitic on birds and mammals (including man). Often they burrow into the skin or greatly irritate it, producing diseases known as itch, mange or scab. The following synopsis includes some of the more important genera:

A. Anal opening dorsal . . . . *Notœdres* — on small mammals
B. Anal opening ventral.
  1. Suckers on legs have jointed stalks
    Psoroptes — on mammals
  2. Suckers with non-jointed stalks or absent.
    a. No suckers on legs of female
      Cnemidocoptes — on birds
    b. Female with suckers on first two pairs of legs, at least.
      a′. Legs very short and stumpy, body short
        Sarcoptes — on man and other mammals.

b′. Legs slender, body more elongate.
a″. Female with suckers on fourth pair of legs . *Chorioptes* — on mammals
b″. Female without suckers on fourth pair of legs.
Otodectes — in ears of mammals.

Some of the commoner species are listed below:

Notœdres cati — causes mange in cats and other small mammals.

Psoroptes communis and varieties — causes Sheep Scab, Texas Itch of cattle, and mange in horses, dogs, etc.

Psoroptes cuniculi — causes Otocariasis in rabbits.

Cnemidocoptes mutans — causes Scaly Leg of fowls and cagebirds.

Cnemidocoptes gallinœ — the de-pluming mite of fowls.

Sarcoptes scabei and varieties — causes scabies in man and mange in various large mammals.

Chorioptes equi — causes Chorioptic Itch in horses and other mammals.

Otodectes cynotis — causes Otocariasis in dogs and cats.

7. Family *Tyroglyphidæ* — Flour-mites.

This family includes a number of small, soft-bodied mites, which infest stored vegetable products of many kinds. Various species may be accidentally transferred from such products to men who handle them, and then give rise to skin irritation and itching called by the names of Grocer's Itch, Copra Itch, Coolie Itch, etc.

B. Order *Araneida*

The Spiders are of interest to medical men only because of the venomous bites inflicted by a few

species. The vast majority of spiders are absolutely harmless, having neither a venom sufficiently powerful to harm a human being nor mandibles strong enough to pierce the skin. Even the gigantic Tarantulas of the southwestern United States and the related Bird-killing Spiders of the tropics seem to be dangerous mainly because of the size of the wound they can inflict and the possibility of secondary infection, rather than because of their venom.

It is certain, however, that certain members of the family *Theridiidæ*, particularly species of the genus *Latrodectes*, in spite of their comparatively small size, have venom powerful enough to produce severe systemic disturbances, sometimes culminating in death, in men whom they bite.

The family *Theridiidæ* is distinguished from other spiders by the following characteristics:

The poison claw of the mandibles is hinged horizontally. The feet have three claws. There are only six spinnerets on the ventral surface just in front of the anus (no broad, unpaired spinneret or "cribellum" is present), and the spinnerets are all short. There are eight eyes in two rows, all about the same size, and they are placed well back from the anterior margin of the cephalo-thorax, the space between margin and eyes being greater than that occupied by the eyes themselves.

The American species, *Latrodectes mactans*, may be encountered almost anywhere in the tropical and subtropical portions of the American continents. The adult female has a body about half an inch long, with legs a little longer. The eyes are dissimilar in color, placed before the middle of the cephalo-

thorax, the lateral eyes of each side widely separated. The tarsus of the last pair of legs bears a single series of curved setæ. The ground-color of this species is coal-black, with an hour-glass-shaped spot on the ventral surface and a series of mid-dorsal spots red or yellow. The male is smaller and has red or yellow lateral spots as well as the dorsal and ventral ones. In the United States this species seems to frequent outdoor privies, and most of the fatalities from its bite have resulted from this habit.

Other species of *Latrodectes* which are feared, probably with reason, in the parts of the world where they occur are:

Latrodectes malmigniattus — the "Malmigniatte" of southern Europe.
Latrodectes erebus — the "Karakurte" of southeastern Russia.
Latrodectes hasseltii — the "Katipo" of New Zealand.
Latrodectes geometricus — in California.

Other species of this genus and related genera should be considered as suspects.

C. Order *Scorpionidea*

All true Scorpions possess the characteristic, slender, segmented "tail," ending in the poison-spine with which they kill their prey, striking, be it noted, only forward over their backs, never to the rear. The identification of the species and higher groups is of no importance to a medical man. Scorpion venom, according to Calmette, produces symptoms of the same kind as does cobra venom,

but of much less severity. While the sting of a large scorpion may produce severe shock, it is not ordinarily fatal.

4. Class Myriapoda

Sharp distinction should be made between the two main Orders of this Class:

1. *Chilognatha* — Millipedes — With two pairs of legs on the majority of the segments.
2. *Chilopoda* — Centipedes — With only one pair of legs to a segment.

The Millipedes are vegetable-feeders and are absolutely harmless. The Centipedes feed on insects and other small animals, and the large tropical species are said to have venom powerful enough to produce severe symptoms in man. The poison-glands open through the large venom-claws on the ventral surface of the head. The severe local inflammation which sometimes follows a centipede bite is probably due to secondary infection. The legs of a centipede may produce infected wounds of this kind, although they have no poison-glands or ducts.

5. Collecting and Preserving Insects

1. Collecting Insects.

Small flies and most insects of other Orders which interest medical men can usually be caught directly into a vial or killing-tube. For flies larger than a house-fly, a net is almost essential. Most of the net frames sold by dealers in entomological supplies are good enough, or a frame can be improvised from

heavy wire or springy wood. In any case, it will usually be necessary to construct the bag yourself from fine gauze, since the bags usually sold, intended for the capture of butterflies, are of too coarse a material to hold small insects.

For fleas, lice, ticks and other ectoparasites, a pair of fine forceps and a small camel's-hair brush are the best implements. A fine-toothed comb is often very useful, also.

For bringing larvæ of flies to the laboratory, a good supply of small, wide-mouthed bottles are advisable. Such bottles or drinking-glasses, with a gauze cover held on by a rubber band, form useful cages for breeding out larvæ of mosquitoes, flies, etc. For collecting the larvæ of mosquitoes and other aquatic flies, a small dipper, preferably white inside, and a wide-mouthed pipette are desirable, while forceps and a small spatula or spoon are a great convenience in searching for muscoid larvæ.

2. Killing Insects.

For killing adult insects, most entomologists use a cyanide-bottle, made by placing several lumps of potassium or sodium cyanide at the bottom of a wide-mouthed bottle and pouring over them a layer of plaster-of-paris, mixed with a small amount of water. When the plaster has set, it is necessary to cover it with several layers of absorbent paper, to keep the insects from becoming wet or soiled.

A neater killing-tube for small insects can be made by placing a few rubber bands or a slice from a small

rubber stopper at the bottom of a large test-tube or vial, and keeping the rubber covered with chloroform for about twenty-four hours. By this time the rubber will have absorbed much of the chloroform and have swollen considerably. The surplus chloroform may now be poured off and a thin layer of cotton introduced and covered with absorbent paper, so that the insects can neither come in contact with the wet rubber nor entangle themselves in the cotton. Such a tube will kill insects for several months, if kept tightly corked when not in use, and it can be renewed at any time by taking out the paper and cotton and soaking the rubber in chloroform again.

The larvæ of *Diptera* should be killed by dropping them into water at a temperature just below the boiling-point, and then transferred to alcohol (of from 70 to 95 per cent) for preservation. Any of the ectoparasitic insects, which are best preserved in alcohol, may be killed by dropping them directly into the alcohol. This is also a good method for mosquito larvæ.

When breeding out mosquitoes and similar forms, specimens of great scientific interest may be accumulated by preserving specimens of the various larval stages, particularly the mature larvæ or their cast skins, with their respective adults.

3. Preserving Insects.

Practically all insects of medical importance except flies should be preserved in alcohol. Fly larvæ

should also be preserved in this way. Adult *Diptera* can also be preserved in alcohol, but for some purposes it is better to keep them dry. Medical men usually collect insects for two purposes, namely, to have a collection of their own for exhibition and reference, and in order to send specimens away for determination. Adult *Diptera* make the best exhibition specimens if they are pinned as soon as possible after death. Such pinned specimens are best kept in a tight, cork-bottomed insect-box, such as are sold by dealers in entomological supplies. The larger flies should be pinned through the thorax with an insect-pin not larger than a Number 3. Mosquitoes and other very small flies may be impaled on the point of a very small pin (usually called "minuten nadeln") which has previously been inserted in a little strip of cork or a disk of cardboard. The bit of cork or cardboard bearing the small pin and the specimen is then pinned into the insect-box on a stout pin (say a Number 5 insect-pin). Only the especially prepared, black japanned, insect-pins should be used. If white pins are used, verdigris is almost sure to collect outside the specimen and discolor it.

Pinned specimens of flies become very fragile when dry, and are not easy to ship. Flies sent off for identification are usually shipped either dry, between thin layers of cotton in a small box or vial, or else in alcohol. If they are sent dry, they should never be corked up in a vial or enclosed in a tight tin box until they are thoroughly dry, and even then it is

best to put a drop of creosote or carbolic acid on the cotton to prevent the growth of mold. When sending mosquitoes for identification, always include both males and females whenever possible, for many species can be identified only by an examination of the male genitalia.

4. Labeling Specimens.

Insect specimens should never be kept or sent away for identification without labels giving at least the locality and date of capture. Such labels are usually placed on the pins of pinned specimens or inside the vials which contain alcoholic specimens. Further notes on the habits or life-history of insects of medical interest are usually of great value, and should be kept in a notebook or given in a letter accompanying specimens sent for determination.

5. Shipping Specimens.

In shipping any insect specimens, pinned, dry or in alcohol, the specimens themselves should be placed in comparatively small containers (specimen-boxes, pill-boxes, vials, etc.), and these should be carefully packed in a strong outer box, using plenty of cotton, crumpled paper, or other padding to keep the small containers firmly in place and separated from the outer box and from each other. In shipping alcoholic specimens, it is best to add a drop or two of glycerin to each vial and to seal the cork with sealing-wax, candle-grease or paraffin, in order to guard against evaporation. In shipping specimens

from one country to another the packages should be conspicuously labeled NATURAL HISTORY SPECIMENS, to avoid trouble with customs.

The author will be glad to determine specimens of insects of medical importance for correspondents in any part of the world. Packages of specimens should be addressed to —

Dr. F. M. Root,
School of Hygiene and Public Health,
The Johns Hopkins University,
Baltimore, Maryland, U.S.A.

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